

nature

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PhD in evolution

EIGHT months ago the Science Research Council (SRC) produced a report on postgraduate education which by the standards which generally prevail in the academic world was decidedly radical. The working party, headed by SRC chairman Sir Sam Edwards, proposed amongst other things that greater emphasis be placed on graduate students taking a wide range of courses, particularly in their first year. A high proportion of broadly based compulsory course work leading to an examination for a Master's degree or equivalent qualification was suggested. The option of continuing for a further two years, the report went on, should only be open to students who had passed the Master's course and demonstrated real aptitude for research. The example of MIT was clearly well to the fore in the working party's deliberations.

The background to this needs little rehearsing. In the 1960s PhDs were being signed up with enthusiasm, not least by the new universities which then proceeded to generate even more PhDs of their own. These days universities no longer recruit, emigration is less of an option, the civil service does not grow in size, so PhDs find themselves applying for a job, somewhat reluctantly maybe, to those same industries to which they might have applied three years earlier. But industry's view of a PhD is by no means one of universal approbation—indeed, those in industry who think that PhDs are of little use to their needs have been getting much of the attention in the last year or two. Small wonder then that SRC decided that some spring cleaning was necessary of the ideas surrounding the PhD.

In the past two months a form of participatory democracy has been in operation. SRC had already received many written submissions on its report, but it decided to take to the road as well so that the report could be discussed more informally in regional gatherings of university science staff and industrialists. That exercise, which took the SRC to Cardiff, Birmingham, Glasgow, Leeds and London, is complete; now the SRC must try to see whether anything like a coherent policy can emerge from the consultative process. The general conclusions that can be drawn seem to run as follows:

- Industry does not speak with anything like a unified voice in its views about the relevance of the PhD. Even within the same company it is possible to find, say, research directors and personnel managers who would differ in their assessment of the value of the degree.

- Whilst most academics support, or at least don't oppose, the idea of more taught courses, the idea that these should be compulsory has had a very mixed reception.

- The idea that the courses should be concentrated in the first year of postgraduate education has come in for some criticism.

- The nature of the assessment at the end of the first year has caused some alarm, not surprisingly amongst students who fear another year of being tyrannised by examinations.

- Many people have expressed doubts about the feasibility of doing a good research project for a PhD in two years rather than three.

Some of these points will undoubtedly result in a softening of the SRC's approach; more than once the council was urged to encourage evolution rather than impose revolution, and such changes as spring from the exercise are likely to emerge slowly over the next decade. For it is not at all clear that the SRC should use the muscle that it has, in the way of discretion over grant money, to force the pace. Maybe the very fact of the report and the chance to air views will have generated enough momentum.

The one thing that the SRC should not soften on, however, is its conviction that there is not a "correct" duration of three years of research for the PhD. A fairly common view amongst academics was that a period of less than three years in the laboratory is inadequate for students to make a research contribution. If the student is being trained to write papers or know more about some particular subject than anyone else in the world, this may have some element of truth in it. But industry does not usually hire its PhDs for either of these reasons; it hires them for their potential to work in a broadly based team. And the PhD who stays in the academic world is going to have plenty of time to develop the necessary expertise later.

Moreover, it is a poor comment on the quality of postgraduate course work if it doesn't make a significant contribution to helping students to be more effective researchers, by pointing them in the direction of key literature, by exposing them to the ethos of research and by allowing them the luxury of sampling different staff members before having to make a final choice. A year of coursework could easily be worth two of research. □



US takes up the issues . . .

The nuclear exporters meet again soon.
Colin Norman reports on the debate now taking place in the United States

NEXT month, representatives of present and potential nuclear exporting countries are scheduled to meet in London for a second round of secret talks aimed at curbing the proliferation of nuclear weapons. With international trade in nuclear goods now estimated to be well in excess of \$1,000 million a year and growing rapidly, and with several particularly risky transactions in the works, the talks are considered by many to be critical for the future stability of the world.

They will take place against a background of growing demands in the United States for extraordinary measures to prevent potentially dangerous nuclear transactions from taking place. Though some of the strongest demands are coming from Capitol Hill, the issue of nuclear proliferation has even been raised in the Presidential election campaign, and some Administration officials are also taking an increasingly hard line in urging the adoption of strict international safeguards to prevent the diversion of peaceful nuclear technology to weapons production.

Two recent developments are, however, likely to be particularly influential in next month's talks. On May 14, the Senate Foreign Relations Committee approved a foreign aid bill containing a little-noticed provision which would cut off all US aid to countries buying or selling uranium enrichment or nuclear reprocessing plants without adequate safeguards. And, in the same week, the Senate Government Operations Committee approved a bill calling on the President to negotiate a "binding agreement" with other nuclear exporting countries to prohibit the sale of enrichment or reprocessing technology to individual countries which do not already possess nuclear weapons. The bill also specifies that the agreement should prohibit the sale of any nuclear facility—including a power reactor—to a country which refuses to place all its nuclear activities under international safeguards and inspection.

Moreover, Senator Abraham Ribicoff, the chairman of the Government Operations Committee, has on several occasions called for strict sanctions against West Germany and France if those countries go ahead with plans to sell reprocessing plants to Brazil and Pakistan respectively.

Thus, with Congress breathing heavily down its neck, the Ford Ad-

ministration is likely to take a tough line in next month's talks. Because some participants in the talks have insisted on strict secrecy—France is said to have threatened to pull out of the first round if the discussions were made public—Administration officials are reluctant to discuss that matter in detail. Nevertheless, it is clear that there is likely to be continuing friction between the United States on the one hand, and France and West Germany on the other.

The first round of the talks, which included representatives from the United States, the USSR, Britain, Canada, France, West Germany and Japan, ended in January with general agreement on principles for international safeguards. According to assorted reports and Administration sources, the agreement would allow nuclear technology to be sold only to those countries which promise not to use it to produce explosives, and it would provide for the adoption of common standards to guard against theft or clandestine diversion of nuclear materials.

The agreement fell far short, however, of the strict controls urged by the United States. The Secretary of State, Henry Kissinger, for example, said in Congressional testimony on March 9 that the talks had simply ended in a "general understanding about restraint", and he suggested that the United States would press for "something more binding" in the next round. The Ford Administration is particularly concerned that the agreement is weak in dealing with the critical issue of the sale of uranium enrichment and fuel reprocessing plants.

Because enrichment plants are capable of producing weapons-grade uranium, and reprocessing plants separate plutonium—another potential bomb ingredient—from spent reactor fuel, they are the key links in a weapons fabrication process. By themselves, reactors present a smaller proliferation hazard since neither the uranium fuel nor the reactor wastes can be used directly to make explosives. Thus reactor sales to non-nuclear weapons countries are usually made on condition that the fuel going into and coming out of the reactor is closely monitored, and that it is not enriched or reprocessed by the purchaser.

The International Atomic Energy Agency (IAEA), the Vienna-based

body established in 1957, is responsible for policing most nuclear transactions to ensure that no diversion of weapons-grade material takes place. IAEA keeps records of fuel passing through reactors under its supervision, and it also has a small corps of inspectors authorised to make spot checks at individual installations. The Nuclear Non-Proliferation Treaty (NPT), which came into force in 1970, binds all signatories to place future nuclear transactions under IAEA supervision.

A number of recent events have, however, shattered hopes that such a loose system of controls can be completely relied upon to prevent the insidious spread of nuclear weapons around the world. The most significant event by far was the detonation in 1974 of an explosive device by India. Using plutonium reprocessed from spent fuel from a Canadian-supplied reactor, India abruptly ended the nuclear hegemony of the United States, the USSR, Britain, France and China. Though Indian officials insisted that they would only use nuclear explosives for peaceful purposes, that piece of semantic juggling does not disguise the fact that India is now a nuclear power.

The other chief unsettling events were the negotiation last year of an agreement between West Germany and Brazil for the sale of an entire nuclear system, including enrichment and reprocessing facilities, followed by a deal between France and Pakistan involving the sale of a reprocessing facility. A third transaction, the sale of a French reprocessing plant to South Korea, was scotched earlier this year when South Korea pulled out under heavy pressure from the United States. (In a press briefing in Washington last



Dr Fred Iklé, Director of the US Arms Control and Disarmament Agency

week, however, the French President, Valéry Giscard d'Estaing, said that he had personally vetoed the sale.) West Germany, in addition, is widely believed to be negotiating to supply reactors, and possibly a reprocessing plant, to Iran.

Those deals mark a significant qualitative shift in international nuclear trade, since they would transfer directly the means for producing weapons-grade materials. What makes them particularly worrying is the fact that none of the recipients, except for Iran, is a party to the NPT. They have all been bitterly opposed by the United States, and US officials from Dr Kissinger down tried in vain to dissuade France and Germany from going through with them.

Aside from sales of enrichment and reprocessing technology, there has also been considerable doubt expressed recently about the adequacy of present safeguards on reactor sales. For one thing, many reactors have already been

sold without IAEA safeguards—the Canadian sale to India provides a particularly vivid example, and similarly the sale by France of a small research reactor to Israel in the early 1960s is widely believed to have provided Israel with the means to produce weapons. According to a recent CIA analysis, Israel has taken up that option and now possesses between 12 and 20 plutonium bombs.

And the IAEA safeguards themselves do not provide an insurmountable barrier against diversion of fissile material. In a speech on May 13, Dr Fred Iklé, Director of the Arms Control and Disarmament Agency (ACDA) and one of the more outspoken Administration officials on nuclear proliferation, warned that the IAEA is understaffed, relies chiefly on information supplied by the country under safeguards, has no power to impose sanctions against violators and cannot investigate unsafeguarded plants. IAEA safeguards, Iklé said, provide “a bur-

glar alarm, but not a lock”, and it is a “fallacy” to believe that we don't have to worry about facilities under IAEA safeguards.

The United States itself, moreover, is not entirely blameless. In 1974, then President Nixon promised to sell nuclear reactors to Israel and Egypt, two antagonists who have not ratified the NPT. (Negotiations concerning those reactors are not yet complete.) And last week it became known that the General Electric Corporation has applied for a licence to sell two 1,000 MW reactors to South Africa, together with 1.4 million pounds of slightly enriched fuel. According to Administration sources, that deal is likely to be officially approved, even though South Africa has not signed the NPT and is believed to be interested in joining the nuclear weapons club.

That is the background against which the talks between the nuclear exporting nations will resume next month. Aside from the seven original participants, they are expected to include representatives from the Netherlands, Sweden, Belgium, East Germany, Italy and Poland. The critical issue is again expected to be the sale of enrichment and reprocessing technology, with the United States arguing against deals which would place such plants in the hands of individual purchasers, whether or not they are placed under IAEA safeguards.

The chief American argument is simply that reprocessing is not necessary at this time, and there is no economic need to sell either enrichment or reprocessing plants. Dr Iklé, for example, noted in his May 13 speech that separating plutonium from spent fuel and recycling it “could replace at most about one third of the fuel required, and far less in a rapidly growing nuclear power system. Hence, recycling would not bring independence from imported fuel”. He added: “Before we plunge into a plutonium fuel economy, let us look very closely at the risks and our ability to control them . . . spreading plutonium should be avoided if possible, and with the current generation of reactors it can be avoided at no economic cost”.

If it proves impossible to curb the desire for reprocessing plants, the Administration will probably continue to urge that instead of selling such plants to individual countries, nuclear exporters should consider placing such technology under multinational control. Iklé noted that the United States is now studying the feasibility of “multinational fuel centres for storage of fuel, waste management, and other services when needed”.

The United States' arguments are, however, likely to fall foul of the desire,

Passage to India?

THE Nuclear Regulatory Commission (NRC) announced last week that it will hold public hearings to decide whether or not the United States should supply slightly enriched uranium to India, for the Tarapur Atomic Reactor located near Bombay. The hearings, the first ever to be held on a nuclear export licence application, graphically underline the fact that because the United States is the world's major supplier of reactor fuel, it is in a strong position to force many countries to accept strong safeguards against the diversion of peaceful nuclear technology to weapons production.

The hearings, set for June 2, will be held in response to a petition from the Natural Resources Defense Council, the Sierra Club and the Union of Concerned Scientists. The United States has supplied India with fuel for that reactor in the past, but the petitioners are essentially suggesting that NRC should use the application to supply another 40,000 pounds of uranium as a way to force India to accept extra safeguards.

The Tarapur reactor and its fuel supplies are all under the supervision of the International Atomic Energy Agency (IAEA), and those facilities were not the source of plutonium for India's nuclear explosives.

But the petitioners have pointed out that India hasn't signed the non-proliferation treaty, that a clash between India and one of its neighbours might disrupt present safeguards at the plant, that the United States has not required India to place all its other nuclear facilities under international

safeguards, and that the United States has not required India to accept bilateral safeguards in addition to the IAEA controls. The implication is that the United States should threaten to shut off fuel supplies to India unless it accepts those additional safeguards. The concept could clearly be applied to other countries.

At present, only the United States and the Soviet Union export enriched uranium fuel, and their dominance of the fuel export market is expected to last at least until the mid-1980s. Some observers have therefore raised the possibility of a joint US-USSR agreement that future fuel supplies should carry strict safeguards agreements, in addition to IAEA controls. Senator Abraham Ribicoff, for example, has even suggested that the United States should refuse to supply nuclear fuel to West Germany and France if those countries persist in selling enrichment or reprocessing plants. But Henry Kissinger has forcefully ruled out such “blackmail”, suggesting that a pact with the Soviet Union against America's NATO allies would have “the gravest foreign policy consequences”.

As for the proposed fuel shipment to India, the NRC has announced that it will decide whether to issue the licence before the end of June, and it has suggested that it might even act on the matter before the conclusion of the hearings “if it finds a need for greater expedition”. The hearings would, however, carry on with an examination of the broad policy issues involved in United States' fuel exports.

particularly on the part of France and West Germany, to earn maximum export dollars from nuclear trade, and of deep European suspicions of American motives. According to some observers, there is concern in Europe that American attempts to prevent the German-Brazil deal, in particular, were chiefly designed to protect the United States' commercial interests there.

Such suspicions were given a blast of fertiliser last year by an incident which occurred just as the United States was applying pressure on West Germany not to conclude its fuel technology agreement with Brazil. According to published accounts, a representative of the Bechtel Power Corporation, a major US nuclear manufacturer, met in March last year with Brazilian officials to discuss the possibility of building fuel facilities there, leaving the impression that the US government would sanction such a deal. Though the incident was probably simply a product of poor communications between industry and government in the United States, it clearly left a sour impression in Europe. Moreover, it should be noted that while Dr Iklé has been arguing that there is no economic incentive for recycling plutonium at present, the US nuclear industry has been urging the government to allow plutonium recycling in the United States.

If the provision in the foreign aid bill survives the rest of the Con-

gressional mill intact, however, it would greatly stiffen the administration's policies in trying to dissuade the sale of enrichment and reprocessing technology. Proposed by Senator Stuart Symington, it would cut off nearly all US aid to the buyers and sellers of such technology, unless they have agreed to "place all such equipment, materials, and technology, upon delivery, under multilateral auspices and management when available", and unless the recipient agrees to place all its nuclear facilities under IAEA safeguards. The latter provision is intended to prevent the recipient from either duplicating the reprocessing or enrichment technology, or separating plutonium from an unsafeguarded reactor in the transferred reprocessing plant. The amendment is not, however, included in the House version of the bill, and its prospects are uncertain.

As for safeguards on the sale of power reactors, the Administration's policy is that controls should be applied uniformly among the supplying countries rather than imposed unilaterally, the argument being that if the United States insists on excessively strict safeguards on its own sales, potential purchasers will simply look elsewhere. Key issues in next month's talks are likely to include ways to bring presently unsafeguarded reactors and facilities into the IAEA safeguards system, methods to ensure that promises not to use imported nuclear technology for weapons

production are made binding, and strengthening of the IAEA safeguards and inspection system.

Aside from the suppliers' conference, Mr Jimmy Carter, the leading candidate to be the Democratic Party's Presidential nominee, has called for a UN-sponsored World Energy Conference to discuss worldwide energy problems and alternatives to nuclear power. Calling nuclear proliferation a "fear-some prospect", Carter also urged a ban on sales of reprocessing and enrichment technology, and he called for a pact among purchasers of nuclear technology to buy only from suppliers who require proper safeguards. "The hour is too late for business as usual", he said. So far, nobody else has talked about those issues in the campaign.

The outcome of the exporters' conference is clearly going to be of immense importance. As Denis Hayes, a researcher with the Worldwatch Institute noted in a recent study on nuclear power, if a few more nations acquire nuclear weapons, there will come a point at which the "dam will break and the world will go nuclear". And, in Congressional testimony earlier this year, David Lilienthal, the first chairman of the US Atomic Energy Commission called for an immediate, unilateral halt to all US nuclear exports because of the dangers of proliferation. "I'm glad I'm not a young man", he said, and "I'm sorry for my grandchildren". □

... Canada makes up its mind

Canada announced last week that further nuclear cooperation with India was impossible.

David Spurgeon in Ottawa gives the background

THE Canadian government is just emerging from some serious soul-searching about the moral, political and economic questions involved in nuclear assistance and reactor sales abroad — particularly to developing countries. In the Canadian House of Commons last week, where the subject has been under debate for some time, the External Affairs Minister, Mr Allan MacEachen, announced that the government had decided that it will not resume supplies to India of nuclear equipment and technology. The two countries have failed to agree on safeguards against the use of the materials supplied by Canada for nuclear explosions.

The decision marks another step in the development of Canadian nuclear policy. The whole subject—which an MP described in one session as "per-

haps the most important ever raised on an opposition day"—originally arose in the House as a result of government negotiations to resume nuclear assistance to India. In March these negotiations were conducted with India in New Delhi by Ivan Head, foreign policy adviser to the Prime Minister, Pierre Trudeau, and Michel Dupuy, an assistant under-secretary in the external affairs department.

This had followed the suspension of assistance after India's explosion of what it called a peaceful nuclear device in May, 1974. The device used plutonium from the Canadian-design CIRUS research reactor, and the suspension had been ordered because, in the words of MacEachen, "the carrying out of that explosion was in clear violation of the understanding that had been reached between Canada and India".

The latest news probably means that the efforts to reach international understandings on nuclear exports have suffered something of a setback. But the Canadian government has meanwhile been pursuing its chosen path in negotiations with other countries. Canada hopes to obtain an agreement with Pakistan not to use plutonium from a Canadian-designed nuclear power plant, and in January concluded agreements to build 600 megawatt power reactors in Argentina and South Korea. An Atomic Energy of Canada Ltd (AECL) spokesman has said there is the prospect of building second units in Argentina and South Korea in future, and preliminary discussions have been undertaken with other countries, including Mexico. Canada is also involved in licensing negotiations with Italy and Romania. Altogether, expected exports of CANDU reactors between 1974 and 1983 have been estimated by the federal department of industry, trade and commerce to amount to \$3,000 million.

The debate over the propriety of Canada's selling nuclear reactors and technology when world powers are con-

cerned about the proliferation of nuclear weapons has gone beyond Parliament. The Prime Minister has received delegations from the Canadian Council of Churches, the United Church of Canada, and the Quakers, and he has been urged to call a halt to the sales. The irony is that the CANDU nuclear reactor has turned out to be one of the most successful in the world at a time when confidence in reactor reliability is waning throughout the world and construction starts are falling off. Canada went it alone with its design (the CANDU is moderated by heavy water and fuelled with natural uranium), and finally appears able, as a result of worldwide interest, to recoup some of its huge investment made over the years.

Critics of the government's policy have expressed fears that CANDU reactors will be used to recover plutonium (produced as a by-product) for the making of nuclear weapons. In the eyes of T. C. Douglas, the former leader of the New Democratic Party, for example, "Canada has greater special responsibility than any other country"; the CANDU reactor, he points out, produces twice as much plutonium as light-water reactors, and spent-fuel rods containing plutonium can be removed daily without shutting down the reactor. On the other hand, Frank Maine, a Liberal MP with a PhD in organic chemistry who is one of the few members of the House with a background in science, has claimed that there are "technical reasons why the CANDU does not contribute to the proliferation of nuclear weapons."

The argument was put in these terms. Since the CANDU is fuelled with natural uranium it converts a lot of its primary fuel component, Uranium 238, into Plutonium 239 and Plutonium 240. Plutonium 239 is used primarily to make bombs, but Plutonium 240 is not very useful for bomb-making. The operating conditions for bomb-making and electricity-generation, however, are contradictory in a CANDU reactor: the longer the fuel is left in the reactor, the more plutonium 240 is made; the shorter the time, the more plutonium 239; but the longer the reactor is left running without changing fuel, the more economic it is. To make plutonium for a bomb, the fuel rods must be left in the reactor for perhaps 600 megawatt days per tonne; but if economic electricity production is wanted, the rods must be left in for 6,000 megawatt days.

Thus, said Dr Maine, the CANDU system is "a total design":

It is designed to be fed the fuel continuously, and the feeding of the reactor is such that the uranium will be irradiated to the 6,000 megawatt days per tonne level.

If you change it by a factor of ten,

either you have to redesign your system completely . . . or else you will have to shut the system down to take out the fuel . . . if you want to generate electricity you use the CANDU system, but if you want to make bombs you do not use the CANDU system.

Dr Maine's technical arguments had little effect on the debates. What bothered many of the opposition members was what they considered a secretive attitude on the part of the government concerning its negotiations with India. Mr Douglas complained that when newspaper reports first appeared about the Delhi delegation and inquiries were made, the Secretary of State for External Affairs "said that the delegation was in India and until it returned he was not in a position to say what agreement it might be discussing". When the delegation did return, "he said the matter was before Cabinet and he could not discuss it until a decision had been made."

Another aspect that bothered critics was that they felt they did not know exactly what was decided at the meetings in London of the "Group of Seven". The Government's position

has been that it cannot in good conscience withhold from developing countries the technology they need to help supply their power requirements. Mr MacEachen has also claimed that Canada has had considerable influence in upgrading nuclear standards among the suppliers, and that if Canada withdrew from the export market it would lose this influence. There would also be domestic consequences, such as a radical dislocation of the uranium mining industry. "If we want to succeed in the task of ensuring non-proliferation, we must cast the net wider", Mr MacEachen told the Commons. "We must consider the causes of international tension and do something about the disparities that exist in the world. We must do something to bring about a better and more equitable international economic system."

In addition to the CIRUS research reactor from which India obtained the plutonium for its nuclear explosive device, Canada has helped to build two reactors known as RAPP-I and RAPP-II. Completion of the second has been held up by Canada's decision not to

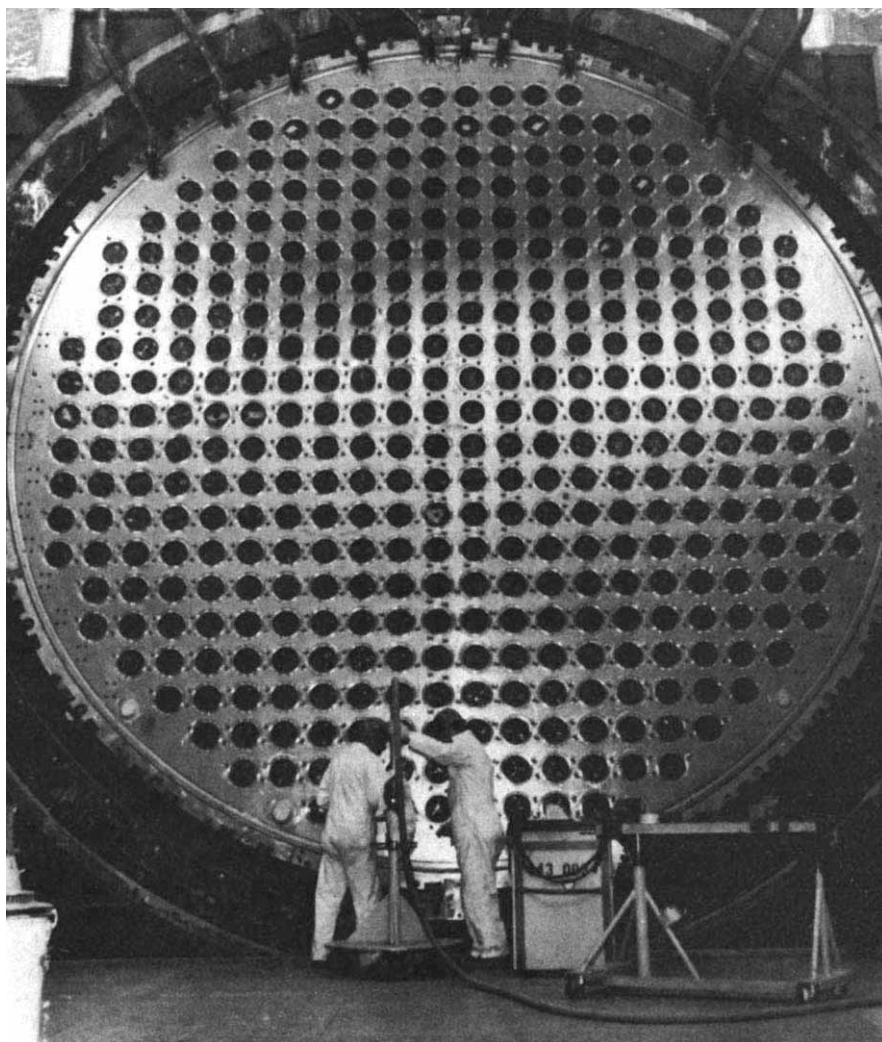


Photo: IPC

What the fuss is all about: part of a CANDU reactor

supply further nuclear assistance without assurance that plutonium coming from it will not be used for further nuclear explosions. It is the lack of that assurance which is chiefly responsible for last week's announcement. The Canadian government has wanted to upgrade the safeguard system which already exists on RAPP-II, which India is capable of finishing alone, though it will take longer without Canadian help.

According to Mr MacEachen, the question the government has been faced with is whether it is better in the interests of non-proliferation to get out of nuclear co-operation with India completely, risking a collapse of current safeguards, or to complete the project while upgrading the RAPP-II safeguards. The crucial point, however, concerns the absence of safeguards on the very reactor that produced India's first nuclear explosion—the CIRUS. CIRUS would not have been covered by the agreement, Canadian officials apparently thinking it unimportant because it produces only enough plutonium to make one or two bombs a year. Furthermore, future reactors now being built or planned by India to Canadian design would not have been safeguarded by the terms of the Canadian agreement.

Before the latest decision the rationale that the government was using

seemed to ignore the very rationale Frank Maine had put forward to defend it: that, because the RAPP reactors are designed to produce power, they could only be used to produce weapons-grade plutonium at the expense of power production. On the other hand, a research reactor like CIRUS is ideal for producing fissile material. That, in fact, is what was done two years ago.

Another question critics asked was how much Canada could rely on safeguards even if they were agreed to. Canada thought it had agreement from India that she would not explode a nuclear device before she actually did so. "Safeguards," said T. C. Douglas, "are worth nothing unless the people who give them are the kind of people on whom we can rely." Robert Stanfield, the former opposition leader, wondered if "the government and those who are advising the government are not acting on the premise that these potential customers are going to get the bomb anyway, and Canada might as well get the business".

There has been some indication that a firm stand by Canada does have some effect. South Korea cancelled a fuel re-processing plant under pressure from the United States and Canada when Canada threatened to cancel construction of its nuclear plant. Pakistan has

contracted with France to buy a processing plant—for reasons unsatisfactory to Canada—and Canada was hoping that if its agreement with India was successfully concluded it would obtain a similar agreement with Pakistan. Such an agreement would prevent Pakistan from using explosive devices from waste fuel from a Canadian-designed plant in Karachi. Until it receives a commitment, Canada will not supply Pakistan with a plant to make fuel bundles for the Karachi reactor, and has threatened to hold back delivery of fuel made in Canada.

What the Canadian nuclear export critics have been saying is that Canada should undertake a moratorium on sales of nuclear materials for a period, during which pressure should be exerted on other exporting countries to agree on adequate safeguards, with sanctions. With respect to India at least, the Cabinet has now made a decision, and through its external affairs minister the Indian government has already expressed its regret and disappointment. The full background behind the Canadian announcement has yet to emerge—India quickly disclaimed any responsibility on her part—and the forthcoming meeting of the "Group of Seven" has now taken on added interest. □

CHEMICAL WEAPONS

Slow progress to disarmament

Attempts are being made to reduce, through international understandings, the chances of chemical warfare. John Stares outlines some of the problems

DRAWING up an international treaty banning chemical weapons ought to be a simple matter. Chemical warfare has been illegal for more than 50 years. No chemical weapons have been manufactured, at least in the West, for almost 10 years, and some countries have begun to dispose of their obsolete stocks. Admittedly a new generation of chemical weapons—the binary weapons—is being developed in the United States, but the US Congress has so far refused to grant funds for their manufacture. And yet, in almost 10 years of international negotiations, the Conference of the Committee on Disarmament (CCD) in Geneva has failed to produce a chemical disarmament treaty.

As time goes on, the need for a chemical warfare (CW) treaty becomes more urgent. Details of the chemistry of CW agent production are

widely known, and there is a growing risk of the proliferation of chemical weapons. Advances in our knowledge of biochemistry, and developments in genetic engineering, bring with them the danger of the development of new CW agents. It may only be a matter of time before the US Congress does grant procurement funds for binaries. If proliferation, the development of new agents or procurement of binaries does become reality, the problems of reaching agreement on chemical disarmament will increase considerably. But in a major speech to the CCD last month, the US delegate, Joseph Martin, indicated that agreement is still a long way off.

Ambassador Martin said that there are several issues where, in the US view, "an adequate basis for forming judgments already seems to exist, and where agreement may be possible in the relatively near future". On the question of the scope of a CW treaty, he said that "the complete prohibition of chemical weapons cannot be realised in a single comprehensive agreement", and that therefore there should be a phased approach to chemical dis-

armament. A step-by-step approach is not a new idea, but while in the past the aim was to concentrate initially on the most dangerous "supertoxic" CW agents, such as the nerve gases, it is now generally agreed that an initial CW treaty should encompass all lethal CW agents.

Thus the phased approach to chemical disarmament should be based on the activities to be included in the ban. Initially attention should be focused on banning the production of all lethal CW agents, and on reducing existing stockpiles, although exactly what measures should be applied to stocks—whether, for example, the treaty should require destruction of all, or only part, of the stocks—is still a matter for debate.

On the question of the definition of CW agents, it is the "tentative view" of the USA that it might be adequate to rely on a general purpose criterion—perhaps along the lines of the formulation in the 1974 Japanese draft CW treaty, where CW agents were defined as chemicals "of types and in quantities that have no justification for protective or other peaceful purposes"—and on lethal toxicity standards. Other criteria, such as representative lists of agents, or structural formulae,

could perhaps be used as well, if necessary.

To have taken five years to reach a consensus that, in an initial CW treaty, production of all lethal CW agents should be banned, that lethal CW agents can be defined satisfactorily, and that some, as yet still unspecified, measures should be applied to existing stockpiles, is not exactly impressive progress—particularly when there is still no agreement on how to ensure compliance with such a treaty. Verification remains the major stumbling block to the conclusion of any CW treaty, partial or otherwise.

In recent years, a mass of detailed information on technical methods of verification has been produced by SIPRI, Pugwash and other groups, and presented in the CCD. It has been suggested that therefore all the elements necessary to build an effective CW treaty are now available, and that all that is needed is a political decision to conclude the treaty. But Ambassador Martin stressed that the USA does not share this view, maintaining that effective solutions to the problems of verification have not yet been found.

A first stage CW treaty would require a verification system capable of providing adequate assurance that CW agents are not being produced, and that the destruction of stockpiles actually complies, qualitatively and quantitatively, with the provisions of the treaty. There are two broad aspects of verification. First, the methodology. The methods studied include, for example, statistical monitoring of data on the production, transport and use of phosphorus and phosphorus compounds, a technique aimed at detecting possible diversion of phosphorus from civilian industry to military uses; and sophisticated scientific analytical tech-

niques capable of detecting organophosphorus compounds in air, water or soil samples (and thus of detecting production of nerve agents), and of monitoring destruction activities, from remote locations. In addition, reconnaissance satellites might be used to observe mothballed production facilities and hence warn of any resumption of activity.

These methods are certainly not foolproof. But, as Martin admitted, 100% effective verification is not necessarily essential: what is needed is a system capable of setting the difficulties of evasion high enough to deter treaty violation. These methods, especially when used in combination, could form a useful basis for such a system. And the methods are constantly being developed and refined: SIPRI, unfortunately, seems to have lost interest in chemical disarmament problems, but other groups, such as the Pugwash Chemical Warfare Study Group, are actively engaged in studies on this question.

The other aspect of verification is the implementation of the system. This is a purely political decision, involving an assessment of the adequacy of the degree of assurance that could be provided by the technical verification methods. Martin made the US view quite clear, that in order to obtain adequate assurance of compliance with the provisions of a CW treaty, there must be provision for supplementing the technical verification methods with on-site inspection of production and destruction facilities, whether on a mandatory or a challenge basis.

On-site inspection has always been a politically sensitive issue, and it is unlikely to be resolved until there is a considerable improvement in international trust and confidence. Of

course, in many ways, this is a circular argument: the greater the degree of international confidence, the easier it would be to implement a verification system including on-site inspection, while at the same time the need for extensive verification would decrease. Nevertheless, there is a point at which the degree of international mutual confidence is such that a given level of verification, including on-site inspection, is both perceived to be necessary, and is acceptable, politically, by all sides.

The international chemical disarmament negotiations therefore seem to have reached the stage where, all the technical elements of a verification system being available, the emphasis is on building international confidence. In this context, Ambassador Martin made the valuable suggestion that it "might be useful to consider the feasibility and utility of technical exchange visits to selected chemical production and disposal facilities in different countries". This was an idea that had been emplaced last year within the US administration, and those of other countries, by the Pugwash Chemical Warfare Study Group. It is encouraging that its public endorsement by Ambassador Martin coincided with the Group's latest Workshop, held in London and attended by experts from 11 countries, East, West and non-aligned.

There have been indications, unofficially, that other countries, including the Soviet Union, would respond favourably to this idea. If a programme of visits could be arranged, at which it would be possible to test the methodology of verification in practice, this would represent a significant step forward in the chemical disarmament negotiations. □

ASBESTOS

Tiny straws in the wind

Recent events in Britain have raised anew questions about the true extent of the asbestos health threat. Allan Piper reports on signs that white asbestos may, after all, be just as dangerous as the blue variety, and that the hazards stretch beyond the risk of asbestosis on the factory floor

THAT asbestos is a threat to health is nothing new—the progressive respiratory disease known as asbestosis led to the first asbestos regulations for industry fully 45 years ago. But the latest controversy in Britain has done more than raise questions about the adequacy of the regulations now cover-

ing its industrial use; it has also served to publicise the possibility that asbestos poses a threat to the public at large.

For asbestos does not just cause asbestosis. It is also a carcinogen. Minute asbestos fibres, if inspired, can pierce individual cells to cause both cancer of the lung and mesothelioma, a particularly malignant, inoperable cancer which attacks the lining of the pleural cavity. There is also emerging evidence that fibres can migrate across internal membranes: they have been detected in cancerous liver tissue, and may also be able to infiltrate the gastrointestinal tract.

It was only at the end of last month that some of the crucial issues became

fully public, when an eight-year-old Department of Health (DHSS) report (together with a revised version) was suddenly made available in the Parliamentary Library following a question in the House of Commons. The report, which was prepared for the DHSS Standing Medical Advisory Committee by a working party it established under the Chairmanship of Sir Richard Doll (then Director of the Medical Research Council's Statistical Unit in London, and now Regius Professor of Medicine at Oxford), clearly states that minimal levels of exposure to asbestos pose a real cancer hazard. According to the report the dangers are not necessarily limited to the naturally fine, blue asbestos, crocidolite, as has hitherto been maintained; they probably extend also to the extensively used white

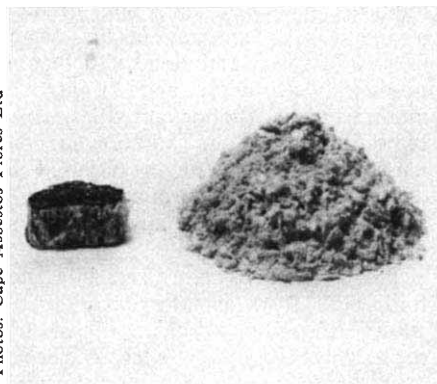
asbestos, chrysotile, which is now milled far more finely than in the past—thus posing a far more widespread threat than previously believed (a possibility which the report clearly acknowledges).

In that light, the official view that the dangers of asbestos have remained confined to industry seems complacent. Even regarding the situation within industry, the suggestion that the cancer threat was removed with the exclusion of crocidolite under the updated Asbestos Regulations of 1969 seems worrying now that the DHSS report has identified chrysotile as a possible hazard. Moreover, the report also called for a review of the issues once the 1969 regulations had been in force for five years. That dateline came up last May, but the review is only now getting under way—and not because of the report itself.

How far the authorities should have moved towards enforcing more stringent regulations remains somewhat arguable, however, if only because the broader scientific community can provide few helpful clues which might finally determine the true extent of the dangers of asbestos. A major difficulty arises because there have been few sufficiently long-term studies. Asbestos fibres can only be detected in diseased tissue using electron microscopy, and the complication means that until now they have been sought only in cases known to be associated with asbestos. Consequently, epidemiological studies—investigations of disease patterns throughout the population—are lacking. In fact, the situation remains so unclear that different interpretations of such data as exist are leading to a rift within the involved scientific community.

Extremes of opinion about the report's claims are easily found. One Medical Research Council (MRC) scientist told *Nature* there was "no evidence at all" of any cancer risk to the general population as suggested in the DHSS report. He was also sceptical of the suggestion that chrysotile may be as dangerous as crocidolite. These rejections came even though a colleague at the same unit has published data showing that of 120 mesothelioma cases in a single study, 23—the largest single statistical unit—were attributable to chance exposure to minimal levels of chrysotile.

On the other hand, a research worker in London fully backed the DHSS report, and even went on to suggest that glass fibres and commercially available talcum powder should also be studied as possible carcinogens. These materials could pose a cancer threat similar to that presented by asbestos since it is the size of the fibres, rather than any chemical agent, which is sus-

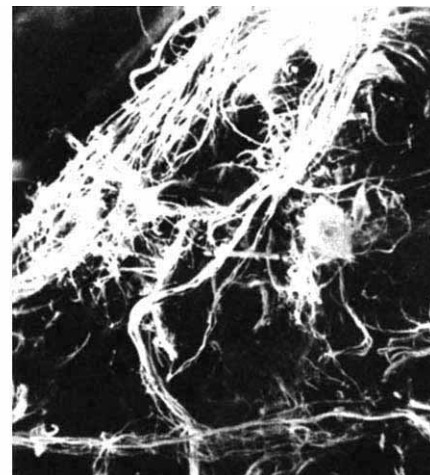


Photos: Cape Asbestos Fibres Ltd

pected of inducing cancer. In fact, a recommendation on minimum fibre-glass diameters has already gone out to industry from one MRC unit. *Nature* was also told that asbestos fibres in the form of anthophyllite have already been detected in studied samples of talcum powder, but a member of the MRC's Pneumoconiosis Unit at Penarth refutes this, claiming that extensive studies on talcs from all over Europe have failed to reveal a single fibre.

The hope is that the ongoing research effort will help resolve the controversy. In Britain work at six MRC units ranges from a major co-operative programme with the Ministry of Defence (into asbestos hazards in naval dockyards), to studies of the aerodynamic properties of fibres. Further MRC laboratory studies of glass fibres are also planned to accompany research already in progress under the sponsorship of the DHSS Health and Safety Executive (HSE), while a major, year-long epidemiological study of all mesothelioma cases should soon be under way at Penarth. In addition, several projects are supported in British universities. On the international front the World Health Organisation's Agency for Research on Cancer announced plans last January for a coordinated epidemiological study on fibre glass workers in Europe; and when its Advisory Committee on Asbestos Cancers meets later this year more research recommendations can be expected.

But while the research efforts continue, the outstanding need for new legislation, and suitable enforcement, remains. The DHSS report underlines the inadequacies of the 1969 regulations, which do not cover the public beyond factory workers and do not recognise the newly defined cancer hazard. Yet no new legislation is planned beyond a marking scheme to cover do-it-yourself asbestos materials. The current review may recommend more, and even the adoption of the



recommendations of the DHSS report would represent a considerable move towards eliminating the general dangers. It would mean the eventual disappearance of crocidolite (which still enters Britain inside manufactured goods), the early introduction of suitable asbestos substitutes, tight controls over the sale, use and disposal of asbestos, and possible checks on general emission levels by the Alkali Inspector.

One difficulty is that the official statistics do not reinforce the case for such action. DHSS figures show only 800 recorded cases of asbestosis, while recorded cases of asbestos cancer and mesothelioma are negligible. But whether the numbers mean very much is questionable on several counts. In the first place, past ignorance of the general danger has ensured that many asbestos cases are attributed to something else (or interpreted as secondary cancers). Industrial settlements out of court provide another distortion. And then there is the hidden factor arising because mesothelioma can take up to 50 years to develop, whereas the asbestos dangers have become significant only during the past 30 or so.

The industry disputes these arguments, and points to a national factory survey by the Health and Safety Executive under existing regulations. Critics regard that move scornfully, particularly after the recent Parliamentary Commissioner's report questioning the vigilance of the Factory Inspectorate at Acre Mill in Yorkshire, the site of an asbestosis tragedy. But they are confronted by a powerful and well-organised industrial lobby, and equivocal union attitudes.

Meanwhile, many unacknowledged asbestos victims (or their families) go uncompensated. Questions about the dangers of asbestos, and about how to minimise them, have been asked and almost forgotten before. At least now it looks as though the latest resurgence of concern could be sustained until answers are found. □

IN BRIEF

Nuclear trade . . . continued

The trends in international nuclear trade were further emphasised by developments last week. South Africa apparently hopes to place the \$1,000 million dollar contract for its proposed twin reactor nuclear power station by the end of the month. A consortium including General Electric of the USA, Brown Boveri of Switzerland and Rijn-Schelde-Verolm of Holland is the leading candidate if credit guarantees can be arranged—a matter now troubling the Dutch coalition government.

Following agreement in principle three months ago in Nice, France and West Germany have now agreed on the practical details under which the French Atomic Energy Commission and the German companies Interatom and Kraftwerk Union will exchange ideas and expertise for a standard marketable design for a fast breeder reactor. Little progress has so far been made on cooperation in the high temperature reactor field, however. Japan-USSR cooperation in the nuclear field,

first mooted in discussions last year, has taken a step forward with the news that Japanese nuclear plant manufacturers are to submit estimates for a proposed Soviet project.

Among other developments: Westinghouse has reportedly signed a letter of intent to sell Egypt one of the two nuclear reactors contained in last year's US-Egypt deal; Kuwait is to seek tenders for a 40–60 MW training reactor as part of its programme for twin 1300 MW stations eventually to provide electricity and desalinated water; and Korea has confirmed that it has not so far approached Britain on nuclear cooperation. Meanwhile, 13 members of the 19-nation International Energy Agency have agreed to exchange information to help insure nuclear reactor safety.

Britain's uranium difficulty

The worldwide scramble for scarce uranium supplies has landed the UK Government in the centre of an embarrassing controversy over a 1970

contract to import an annual 1,500 tonnes of uranium from Rio Tinto Zinc's Rossing mine in the politically-sensitive territory of Namibia. Under strong UN pressure to cancel the deal, the Labour Government is also burdened by a promise to withdraw which it made while in opposition during 1973. Namibian uranium is arguably crucial to Britain's nuclear future: projected 1980 requirements for the UK's first and second generation reactors stand at over 2,000 tonnes a year, and on this point the debate concerns whether, in the face of fierce international competition, UK demands can be met as cheaply or reliably by alternative producers.

Canada's energy moves

In a drive towards energy sufficiency by 1985 Canada is to introduce incentives to boost exploitation of gas and oil reserves, change its licensing system and give the state-owned company Petro-Canada preferential powers over other companies.

ALEXANDER Solzhenitsyn is generally admired as a writer, and as the winner of a Nobel Prize for Literature. His political views are more controversial. In Western countries some of those who have watched his recent interviews on television say they want action at once, to combat the dangers of Soviet imperialism and communist infiltration; others think his views exaggerated and irrelevant to democratic countries. His novel *The First Circle* deals with the political situation in Russia under Stalin in 1950, but it also contains something which may be relevant to the debate about the organisation of scientific research, a debate which is continuing today in Britain, in many European countries and in North America.

The book describes the Mavrino Special Prison, which is in effect a government research laboratory. Many of the inmates are distinguished scientists and technicians who are alleged to have collaborated with the Germans when prisoners of war. In addition there are "free" workers, mostly in junior positions. The establishment is under the direction of scientists who have not yet incurred the displeasure of the regime, with close supervision from government ministers with more or less scientific experience. The purpose of the research is mostly highly practical, generally aimed at improving the surveillance of those suspected of ideological deviation, though sometimes the research workers may

become involved in scientific problems and forget how their results may be applied. It is generally understood that any prisoner whose work is successful will have his sentence

Organised research**KENNETH MELLANBY**

remitted; this is intended to ensure his "motivation".

Much of *The First Circle* concerns politics and the problems of insecurity and intrigue in Stalinist Russia, and is thus not, we hope, directly relevant to laboratories in other countries. Yet the organisation of the research has, in some instances, a disquieting resemblance to the system now being developed in Britain and elsewhere—though in most cases the resemblance is that of an obscene caricature. How-

ever, such caricatures have sometimes given a foretaste of future developments.

Thus at Mavrino we see bureaucracy encroaching everywhere, and an obsession with paperwork. Instead of getting down to actual research, the organising scientists are concerned with "plans, plans, promises and more plans". The scientists as a whole have little influence on the choice of the subject to be investigated, and are often compelled to devote their efforts to topics they know to be worthless. The research programmes are controlled by rigidly described "projects" with an exact—and often impossible—timetable for their completion. When, as is often inevitable, the required results are not forthcoming, a crash programme (described by one of the participants as "heads down and charge") is mounted. As a result we read of "the rushed, sloppy, careless style of work which was the rule" at Mavrino. The whole system is seen to be remarkably inefficient, unable to produce even short term results.

Notwithstanding (or even because of) Solzhenitsyn's gloomy predictions, we may avoid the political fate he warns us against. But I think we can also learn from his account of Stalinist scientific research. Changes in the organisation of science in Western countries seem to be introducing many of the methods used at Mavrino. Can we be sure, even under a democracy, that they will not reduce even further our scientific originality and productivity?

correspondence

Jukes on food

SIR,—Thomas H. Jukes, writing on nutrition (May 13, page 92), tried to be funny at the expense of accuracy. To give but one example, his response to the recommendation from the Royal College of Physicians' Working Party that less butter should be eaten, is that it is "just as logical" to place a warning on "every nubile woman, for human milk contains fats similar to those in cow's milk and we are told the pre-arteriosclerotic changes can start at a very young age".

Really, Mr Nature, this sort of stuff will not do. Cow's milk is suited to body growth, with three times as much protein and mineral as human milk which, by contrast, is suited to brain, vascular and organ biochemistry. Human milk contains between 4–7 times the amount of essential polyunsaturated fatty acids of 18 carbon chain length, and some 8–12 times the amount of long-chain polyunsaturated fatty acids (C20–C22), which are the principal essential fatty acids found in the brain, artery, endocrine and reproductive systems of the body. Perhaps Thomas Jukes had not noticed that the brains and arteries of the human are somewhat differently developed as compared with those of the cow.

M. A. CRAWFORD

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Government and industry

SIR,—Since you have dealt editorially (April 29) with the difficult question of salary comparisons between industry and the civil service, I would seek space to make only two points in reply to Harold Turner (April 8, page 472).

First, I agree with him that the problems of British industry are not primarily technological, but economic and managerial. My article (February 12, page 436) was intended to be a constructive contribution to solving these more serious problems. Second, the CBI has devoted considerable thought to defining the optimum relationship between government and industry in the field of research and technology, and last October sent a fairly lengthy, and generally well-received, document on the subject to appropriate parts of the government machine.

There is no simple formula, but essentially the CBI sees the need to develop government–industry techno-

logical relations through such mechanisms as the Requirements Boards of the Department of Industry, and to establish links between these Boards and the Economic Development Committees which operate under NEDC in order to relate technological to economic factors.

D. W. BUDWORTH

Confederation of British Industry

Isolative sound-change

SIR,—Your correspondents Alan Ross (April 22, page 664) and Ian Hurrell (May 13, page 93) discuss the problem of vowel-shifts with particular reference to the evolution of the English language. We must first of all be certain that a problem exists.

"Anglo-Saxon" is the name given to the miscellany of people who migrated to the British Isles from all of the coastal regions around the North Sea—from what is now Flanders to northern Scandinavia. The languages and dialects of these peoples, which fused to become the dialects of the Anglo-Saxon language, were related but diverse. Both Anglo-Saxon and Middle English were highly heterogeneous languages, even when only the literary forms are considered (compare the language of *Beowulf* with that of the Exeter Book in the Anglo-Saxon, or compare the original language of *The Canterbury Tales* with that of *Piers Ploughman* or of *Sir Gawain and the Green Knight*, all three of which were written in the late fourteenth Century).

As England became united and contact between dialect groups increased, some words from each dialect would survive but most would vanish, a process that has continued to this day. The literary language at any one time was a reflection of the region of medieval England which was dominant at that time. Either of these two factors would give the impression, judging only from the literary forms which have survived, that well defined shifts in pronunciation had taken place, whereas the changes that did occur were due to chance.

In order to show that a problem in "psycho-linguistics" exists and that the explanation is not trivial, one must show that:

- all dialects of the language, not merely the literary form, changed in the same direction at the same time
- all words in any one dialect with a particular vowel sound changed to another sound (for example, $\bar{a} \rightarrow \bar{o}$)

● these changes were not related to political changes, especially with respect to the region of origin of the royal household.

JOHN J. PRATT

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Tropical trees

SIR,—May suggested (*Nature*, 257, 737–8; 1975) that "relatively stable tropical environment(s)" tend to produce plant species of high competitive ability (K-selected) as opposed to opportunists (r-selected species) which he considers are favoured by "relatively vagarious, temperate climates". This generalisation is misleading. It overlooks the class of trees, also well-represented in tropical rain forest, known as pioneers which are opportunists. These are the species which colonise gaps. They possess a whole syndrome of features which equip them to this niche in the ecosystem.

In brief, they possess small, easily (wind or animal) dispersed seeds which have dormancy and are produced more or less continuously; are extremely fast growing, unable to regenerate under shade, and have pale wood of low density. Balsa (*Ochroma lagopus*) of Latin America is the classic example. Appreciation of the existence of two classes with strongly contrasting biology is vital to an understanding of both the ecology and silviculture of tropical rain forest. For example, it has been realised for some time that the most valuable African rain forests in which timber species of Meliaceae (African mahoganies) dominate are a late seral stage, not replacing itself but altering to a forest with less valuable and smaller trees of different families; and the *Campnosperma* and *Terminalia* forests which are the main natural resource of the Solomon Islands are stands of near-pioneers.

Pioneer species are the ones foresters nowadays mainly plant. Their biology and timber properties suit modern conditions. The humid tropics are well-suited to rapid timber production and in fact tropical pioneer trees will be one of the world's main timber sources by the turn of the century. It is therefore important to correct any misconception that there are no pioneers in tropical rain forest.

T. C. WHITMORE

University of Oxford, UK

news and views

Viral infection mimicking genetic patterns

from Arie J. Zuckerman

THE recognition of hepatitis B surface antigen (originally known as Australia antigen or Au antigen) and its homologous antibody as two specific markers of infection with hepatitis B virus resulted in considerable progress in the understanding of the epidemiological, clinical and immunological behaviour of this infection. A major public health problem that has emerged from the results of numerous surveys is the remarkably high prevalence of persistent carriers of the hepatitis B surface antigen in many areas of the world, resulting in an estimate of over 110 million carriers. For practical purposes, a persistent carrier had been defined as an individual in whom the surface antigen has been detected repeatedly for more than three months. The duration of carriage may be many years (Zuckerman and Taylor, *Nature*, **223**, 81; 1969) but some carriers may lose the antigen after a shorter period of time (Szmuness *et al.*, *Am. J. Epidemiol.*, **98**, 104; 1973).

The reported percentage of carriers varies widely in different geographical areas, from less than 0.1% to as many as 20% of the apparently healthy population. The carrier rate is higher in warm-climate countries than in the temperate zones, higher among males than females, higher among children and adolescents than among adults, and higher among urban than among rural communities. The underlying mechanisms for the establishment of the persistent carrier state have remained largely unknown, although the subject of many investigations. Blumberg and his colleagues (*Proc. natn. Acad. Sci. U.S.A.*, **62**, 1108; 1969) proposed that susceptibility to persistent asymptomatic carriage of hepatitis B surface antigen was based on a simple autosomal recessive gene (Au^1), once infection with hepatitis B virus had occurred. This gene was considered to be common in tropical areas but rare in temperate zones. Individuals homozygous for this gene (Au^1/Au^1) would have persistent antigen without overt manifestation of hepatitis, although they remain as carriers of the associated virus, whereas heterozygotes (Au^1/Au^0) and individuals without the gene (Au^0/Au^0)

would not remain as carriers. This hypothesis has evoked considerable controversy (*Nature*, **248**, 377; 1974). Data now obtained by Stevens and Beasley (*Nature*, **260**, 715; 1976) and Mazzur (this issue, page 316) are not compatible with this simple genetic hypothesis. Stevens and Beasley determined the rates of hepatitis B surface antigenaemia among children in Taiwan, which is a high prevalence area, in families with two antigen carrier parents and compared the rate with those with a carrier mother and an antigen-negative, antibody-positive father. The results suggest that all the children could become antigen carriers, regardless of the genetic background. Furthermore, acquisition of the carrier state was probably influenced by the dose of virus received by the babies (Stevens *et al.*, *New Engl. J. Med.*, **292**, 771; 1975) and the age of infection (Szmuness and Prince, *Am. J. Epidemiol.*, **94**, 585; 1971). From Mazzur's data on the children of matings between two carriers or between one carrier and one non-carrier the genetic hypothesis can also be rejected. In this study there were, for example, three families in which both parents were antigen carriers, yet only 2 of the 13 children in this group were carriers. Three of the children had antibody and therefore they had been infected with hepatitis B virus but they did not become carriers, which is clearly incompatible with the genetic theory. The remaining eight children had no serological evidence of infection. All four children in another family were carriers, although the antigen was not detected in either of the parents. The probability of this occurrence according to the simple genetic hypothesis was calculated as 1 in 256.

More complex genetic explanations have also been proposed, including a link between heterozygosity for Gm antigens and increased susceptibility to persistent antigen carriage. Homozygosity for Gm antigens on the other hand leads to an increased probability of developing surface antibody when infected with hepatitis B virus (Blumberg *et al.*, *Nature*, **236**, 28; 1972). This aspect was not supported by the in-

vestigation of Schanfield *et al.* (*Nature new Biol.*, **243**, 81; 1973) in normal blood donors. In a subsequent multi-centre study, Schanfield and associates (*Int. Symp. Viral Hepatitis. Devl. biol. Standard*, **30**, 257-269, S. Karger, Basel, 1975) found no association between the Gm phenotype and susceptibility to hepatitis B virus, using the surface antigen or the ability to make antibody to it as a marker. It was concluded that variation in the distribution of Gm haplotypes did not seem to be a feasible explanation for variation in surface antigen and surface antibody frequencies in a series of multiply transfused populations in the United Kingdom, Cyprus, Greece and Sardinia.

Another possible genetic association has been described more recently, however. Boettcher and colleagues (*J. Immunogenet.*, **2**, 151; 1975) established an association between an HLA antigen and the presence of hepatitis B surface antigen in a group of Australian aborigines. There was a statistically significant deficiency of W 15 among carriers of the antigen and this was explained in terms of linkage disequilibrium between W 15 and an immune response gene involved in clearing the surface antigen from the body. Although it was pointed out that the hypothesis that becoming a carrier is largely genetically determined is an oversimplification, it was accepted that the potential for becoming a carrier is a recessive condition controlled by a single autosomal gene. It was proposed that if the antigen W 15 is a (hepatitis B) virus receptor on cells, and it is disadvantageous to have viruses located on these receptors on certain cells, it would be of selective advantage also to possess the immune response gene responsible for clearing viruses from the receptors. In these circumstances, association of W 15 and the hepatitis B surface antigen immune response gene would be of selective advantage. Indeed, the major complex of histocompatibility antigens has been shown to be closely associated in animals and in man with the immune response genes. This could explain the association of immunological diseases with HLA antigens, for example on the basis

of a possible receptor role of HLA antigens or the "molecular mimicry" between viruses and HLA antigens. Vladutiu (*Lancet*, ii, 288; 1974) pointed out, however, that lymphocyte-defined determinants have been shown to exhibit a stronger association with diseases than serologically-defined determinants. He also noted that the enthusiasm generated by the idea of association of HLA antigens with various diseases is not unanimous, and although there is a strong association with a few diseases, the underlying mechanism remains unknown. Vladutiu stressed that there are many pitfalls when searching for such associations and that the principal source of errors is the statistical analysis. Another example cited is the association of many diseases with HLA 1 and HLA 8. Although these antigens are absent in Japanese, diseases known to be associated with them are seen

among the Japanese population. It is apparent, therefore, that further studies and experimental models are necessary.

Finally, it is widely recognised that family clustering may be entirely a function of an increased risk of exposure to an infecting virus rather than genetically determined susceptibility; particularly if a parent is excreting the virus and more so if the mother is the transmitter. Mazzur notes that persistent infection with hepatitis B virus may be representative of other infections which may also mimic genetic patterns. Indeed she suggests that perhaps polygenic birth defects and other diseases, which the currently accepted as genetically determined because of family clustering may also be caused by perhaps as yet unrecognised infectious agents. This suggestion merits considerable further investigation and careful evaluation of the results. □

cattle should no longer be needed and basic research will not necessarily have to be done in Africa. This should please the growing number of scientists who are concerned about excessive animal experimentation.

Diseases caused by *Theileria* are not confined to Africa and in India considerable progress is being made in the understanding of the biology of *T. annulata*. Bhattacharyulu, Chaudri and Gill (*Parasitology*, 71, 1; 1975) have confirmed that *Hyalomma anatolicum* and other ticks transmit this disease, that the infection passes from larvae to nymphs and nymphs to adults and that the infection is transmitted during the first 24 h after feeding. Of considerable interest is the finding that if infected larvae are fed on cattle as nymphs the adults are not infective whereas if the nymphs are fed on rabbits which are not susceptible to infection with *T. annulata* the adults are infective. It seems as if the infective stages are "used up" during feeding on an appropriate host. This may call for some reappraisal of the experimental procedure of using "susceptible" and "non-susceptible" hosts almost randomly in various fields of research into piroplasmiasis. The use of cultured parasites would avoid this problem. □

Theileria in the laboratory

from F. E. G. Cox

THE increasing problem of protein shortage in Africa has once again concentrated attention on the parasitic diseases of cattle and although recent attempts at vaccinating animals against these diseases give grounds for optimism (*Nature*, 260, 380; 1976) much basic research still remains to be done. East Coast Fever, caused by a protozoan *Theileria parva* and transmitted by a tick *Rhipicephalus appendiculatus*, still kills up to 40% of calves over a wide area of Central and East Africa. The parasite develops through two cycles of multiplication in lymphocytes before entering red blood cells and becoming infective to the tick. Only cattle, buffalo and Indian water buffalo can support the infection through to its final infective stages and research has been limited by the non-availability of a suitable laboratory host. In 1970 some progress in the maintenance of *T. parva* *in vitro* was made (Malmquist, Nyindo and Brown, *Trop. Anim. Hlth Prod.*, 2, 139; 1970) when it was shown that the early stages of the parasite in lymphocytes could be grown in a bovine cell line but the infection did not proceed through to the blood forms. More recently the use of interspecific heterokaryons formed by the virus-induced fusion of cells has found favour (Irvin *et al.*, *Int. J. Parasitol.*, 4, 519; 1974) but still no invasion of blood cells has been recorded.

In this issue of *Nature* (page 311) Danskin and Wilde describe how they have been able to grow all stages of *T. parva* up to the blood forms in

culture. Their technique is simple; they use the modified Eagle's medium described by Malmquist *et al.* with 20–30% foetal calf serum (instead of 10%) and in addition 5–10% bovine lymph. The usual macroschizonts and microschizonts developed and bovine blood cells added after 18–66 h became infected but not those added after 90 h. The number of cells infected, 0.1%, was low but cattle are infective to ticks at this level of parasitaemia.

The next stage will be to infect ticks from cultures and considerable progress has been made on an understanding of the feeding biology of *R. appendiculatus* by Tukahirwa (*Parasitology*, 72, 65; 1976). The larvae and nymphs require 8–9 d after hatching or moulting before they can feed and this they do for 4–6 d. The adults cannot feed for 6–9 d after moulting but eventually feed for 7–9 d. On *Theileria lawrenci* (a parasite closely related to *T. parva*, so realistic comparisons are possible) adult *R. appendiculatus* infected as nymphs become infective to cattle 3 d after beginning to feed and remain so for the next 6 d (Young *et al.*, *Parasitology*, 71, 27; 1975). These authors were able to correlate infectivity with morphological criteria of parasite maturity in the ticks' salivary glands so it should be possible to infect nymphs from cultures, allow the nymphs to moult, let them feed on rabbits and collect infective stages to initiate further cultures after 3–4 d using the morphology of the parasites as a guide to infectivity. Experimental

Of gods and men

from David W. Hughes

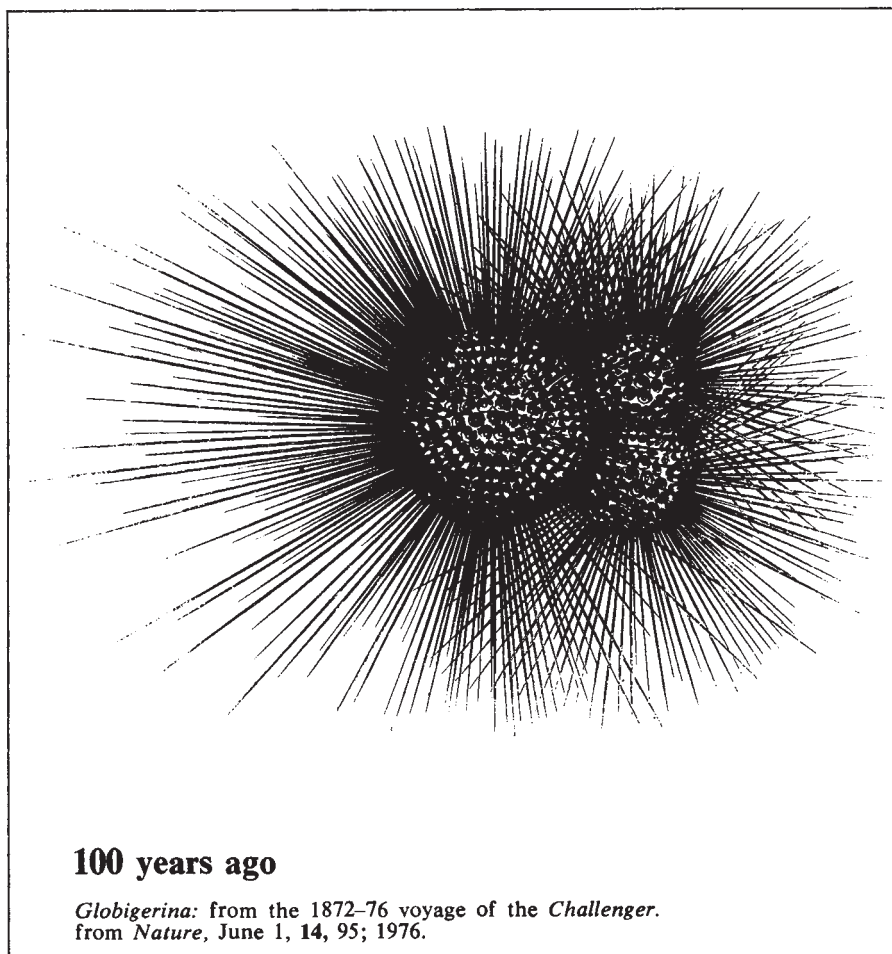
STARS were first named by the herdsmen, hunters, sailors and travellers who lived along the banks of the Euphrates and Ganges, and it was these people and not the learned and scientific who were the sideronomists. The star names used today have been gradually accumulated over the last three thousand years or so. They have come in the main from Greece, Rome and the nomadic and scholarly tribes of Arabia. Castor and Pollux (α and β Geminorum)—the heavenly twins; Regulus (α Leonis)—the ruler of the affairs of the heavens; Betelgeuze (β Orionis) from *ibt al jauzah*, the armpit of the central one, Algol (β Persei) from the Arabian's *Ra's al Ghul*, the Demons head, are a few examples.

Today naming stars has gone out of fashion and astronomers make do with rather dry titles such as V1057 and L789-6. However naming say, Jovian satellites and the features of planetary surfaces that have recently been revealed by imaging devices on space craft is very much in the news and a subject of some controversy.

Carl Sagan (Laboratory for Planetary Studies, Cornell University) discusses this problem in his recent paper in *Icarus* (27, 575; 1976). In 1967, con-

fronted with enormous amounts of new data relating to the near and far side of the Moon, Commission 17 of the International Astronomical Union set up a subcommittee on lunar nomenclature. They decided to extend the previous system of nomenclature to the reverse side of the Moon and to name craters after deceased scientists, special attention (and presumably preference) being given to astronomers. It was also proposed (*Trans. IAU, XIII*, 103; 1967) that the nomenclature should be widened to include people associated with the present space effort and particularly cosmonauts and astronauts and others who have lost their lives in space vehicles. It was also thought that named craters should be at least 18 km in diameter. Conservatism and caution were stressed, the committee members pointing out that the names would be permanent, so only names of permanent renown should be used, revision and reassignment being thought undignified. Features below the minimum size were to be designated accordingly to the Mädler system, capital Roman letters being used for craters, valleys and other depressions, lower case Greek letters being used for hills, mountains and other eminences. In 1973 (*Trans. IAU, XVA*, 205; 1973) the working group went so far as to suggest that small features with diameters less than 100 m should have "given names" such as John, Mary and Nikolai, followed by the name of the nearby major feature. Scientists whose names were similar in sound to others and so omitted earlier were now to be given craters. So Born who had not been included because of his similarity to Bohr, Rutherford—Rutherford, Lawrence—Lorentz, were now in luck. In 1973 the qualification for a place in the nomenclature was also broadened but still the working group emphasised that they would "exclude all political figures, national heroes, religious figures, and modern philosophers. Ancient philosophers and various legendary figures will be accepted, however". The people responsible for Martian nomenclature (*Icarus*, 26, 85; 1975) broadened their outlook still further bringing in biologists, geologists, atmospheric physicists and even science fiction writers concerned with the lore of Mars. The major sinuous channels were named after the name of Mars in a variety of other, largely non-Indo-European, languages.

Sagan advocates that the craters of Mercury should be named after great poets, authors, composers and other scholarly or artistic figures who were not physical scientists. He puts forward a large list of candidates containing such worthies as Dostoyevsky, Bertrand Russell, Picasso, Tchaikowsky and Hiram Bingham. The end result of the



100 years ago

Globigerina: from the 1872–76 voyage of the *Challenger*.
from *Nature*, June 1, 14, 95; 1976.

nomenclature should be a non-provincial distribution of nationalities, epochs and occupations; to quote Sagan "a distribution that our great-grandchildren can be proud of. It is just conceivable that some of them will be living in the places under discussion".

In the more specific cases of the satellites of Jupiter the IAU Working Group for planetary nomenclature have proposed a set of names for satellites V to XIII (*IAU Circular*, No. 2846; 1975). The Galilean Satellites are named after lovers or consorts of Zeus, the father of the gods in Greek mythology. The nomenclature group have tried to continue this tradition, naming the remaining satellites after friends of Zeus (all human, with the exception of Amalthea who was the goat which suckled the infant god). Sagan points out that the names proposed for VI, Himalia, and XII, Ananke don't fit the scheme, Ananke's being the mother of the Fates placed in judgment over the Olympian gods and not a friend of Zeus at all. Also Elara (VII), Lysithea (X), and Carme (XI) are thought, by Sagan, to be a touch obscure. He also finds it surprising that the most prominent remaining consorts seem to have been omitted and suggests that Maia, Leto, Semele, Demeter, Alcmena, Hera, Latona, Aegina, Pasi-

phae, Themis, Metis and Eurynome should be afforded due consideration.

The best chance for a place in the nomenclature for mere mortals, however, still remains the discovery of a comet or asteroid. □

Plant pathology changing direction?

from M. S. Wolfe and J. A. Barrett

A conference on the Genetic Basis of Epidemics in Agriculture was held in New York on April 5–8, 1976 under the auspices of the New York Academy of Sciences.

IN 1970, disaster struck the American corn crop in the form of southern corn leaf blight, a fungal disease which reduced the total yield by 15% and thus rocked the world grain markets. In the same year, and for similar reasons, another cereal pathogen caused much greater suffering, but was less publicised: downy mildew of pearl millet in India reduced the yield of the "poor man's crop" by 30% and directly caused hunger and starvation. The similarity between the two disasters was the development of advanced forms

of hybrid varieties which, in each crop, were dependent on a single type of male sterile cytoplasm and this provided the perfect uniform environment for the explosive development of the two pathogens.

One result of the corn epidemic was the instigation of an American survey of crop uniformity around the world, to determine the extent to which modern methods of agricultural production might be increasing the vulnerability of food production to pest and pathogen epidemics. Superficially at least, the result was alarming in that not only do the world's major crops comprise very few species, but that within each of these species production is dominated by very few varieties, which may often be related to each other.

The meeting was held to assess more critically the risk to world food supplies through the relationship between crop uniformity and disease and pest vulnerability, and the kinds of solutions which could be readily utilised. The general consensus was that since 1970, crop uniformity had, if anything, increased. In the developed countries, this was largely the result of demands by producer, processor and consumer for uniform products. In the developing countries, the main influence was the widespread introduction of breeding material from centralised sources which, at least in the recent past, has had a limited genetic base, and which squeezes indigenous material out of production, thus reducing available genetic resources.

In a comprehensive survey of the evolution of disease problems in tropical crops, I. Buddenhagen (International Institute for Tropical Agriculture, Ibadan) pointed out that increases in crop uniformity did not necessarily lead to problems, and that control in some cases could be effected by straightforward programmes of breeding for resistance. For example, downy mildew of sugar cane in Australia had been eliminated in this way, and in a similar way in Britain, it seems unlikely that eyespot disease of cereals will ever be a very serious threat because of the background of resistance built into cereal varieties grown in the United Kingdom.

For many diseases, however, notably those caused by the leaf pathogens of cereals, increased uniformity of crop production does place us on a knife edge with regard to disease and pest vulnerability. The problem was graphically illustrated by K. M. Safeeulla (University of Mysore) in relation to past and potential problems with the cereal varieties of the Green Revolution introduced into India. The core of the meeting was concerned with this problem and quickly revealed the wisdom

and foresight of the organisers, P. R. Day, J. G. Horsfall and Otto Frankel, in bringing together those who are showing increasing interest in the genetics, epidemiology and mathematical interpretation of the dynamics of the host-parasite interaction as it occurs in the field. As with the early fusion of any range of scientific disciplines, opinions were many and varied, but two general trends could be discerned. First it was likely that there was no universal answer for dealing with all host-parasite problems: different strategies would be optimal for different situations depending on the balance between the level of disease risk and the price that plant breeders and growers are willing to pay to contain that risk. For example, the complete turn-away from crop uniformity by employing mixed varieties or multilines, which are heterogeneous for disease resistance and other characters, may be of advantage only where there is severe and continuous disease present. Second, there was a general, but not unanimous, movement towards the conclusion that no form of host resistance or its manifestation could produce an endlessly stable situation. The aim should be to slow down the rate of pathogen or pest response to the control measures so that it becomes virtually imperceptible, whilst maintaining constant vigilance.

The consideration that the host-parasite interaction could be stabilised, by the exploitation of so-called "horizontal resistance", was argued by R. Robinson (FAO, Rome). "Horizontal resistance" is defined as resistance in the host to all genotypes of the pathogen and Robinson suggested that techniques currently being used in an FAO programme under his direction could accumulate such resistance and thus provide permanent protection. Although some of the techniques have considerable practical value, we remained unconvinced by the theory, particularly since the weight of emerging experimental evidence moves us inexorably towards the view that specific qualitative or quantitative variation exists to correspond with all of the host resistances which have been thoroughly investigated. It does not necessarily follow that all of this variation will be of practical significance.

For all the tension which the pathologists and breeders feel with regard to the vulnerability of major crops to diseases and pests, and for all the differences of opinion experienced over the means of relieving that tension, there emerged from the meeting an underlying feeling of excitement and optimism. Plant pathologists have for too long been following behind the disasters, explaining why the patient suddenly became ill, or patching him up with pesticides. In recent years

however, attention has been increasingly focused on positive strategies of confronting pathogens and pests with increasingly complex problems, based on sound genetical and epidemiological principles. The organisers of the meeting should feel well satisfied in providing an important signpost in this essential direction. □

Plant production in Pakistan

from D. Boulter and A. Muhammed

A Symposium on Genetic Control of Diversity in Plants was held in Lahore from March 1-6, 1976, organised by Dr Amir Muhammed, Vice-Chancellor, University of Agriculture, Lyallpur, and Dr A. Hollaender of the Associated Universities Inc., Washington, DC and sponsored chiefly by the US National Science Foundation.

THE initial promise of the introduction of new dwarf varieties of cereals and the impact of the Green Revolution have not been sustained in Pakistan; agricultural productivity remains disappointingly low. The Government of Pakistan recognises the first importance of agricultural improvement and has provided funds for buildings and facilities. The Punjab Finance Minister, Dr Abdul Khaliq, pointed out that Pakistan is "doomed", as he put it, to be an agricultural country; the only way out is by recognising the value of research which can bring about the revolutionary changes necessary to improve the general prosperity of the country. Thus, although primarily an agricultural country, only 35% of the gross national product derives from agriculture. Time is of the essence since every 5 minutes an acre of agricultural land is lost because of increased salinity. One major constraint on the improvement of agricultural productivity, is that Pakistani scientists lack channels of communication. Their work is often not recognised among their colleagues, and generally stimulation is lacking. So the Symposium was particularly welcome.

It was organised to provide a forum on the latest developments in the field of crop improvement, both fundamental and applied, including those relevant to the adverse conditions of high salinity and aridity.

O. H. Frankel (CSIRO, Canberra) warned of the drastic reduction in the diversity of crop plants brought about by widespread use of new varieties;

Pakistan with its gene centre of primitive wheats and fruits was an important case in point. The exploration and collection of genetic materials for future breeding programmes and the long-term conservation of seeds, are urgently required. S. A. Qureshi (Punjab Agricultural Research Institute, Lyallpur) made a strong case for the use of varieties developed from local programmes instead of selections from exotic materials, since the latter required inputs, particularly fertilisers, which were not being used effectively probably due to inadequate price incentives.

R. D. Brock (CSIRO, Canberra) stressed the important role of mutation breeding which is much underestimated at present; the best immediate prospects for increasing its specificity lie in the manipulation of the selection environment.

The development of high yielding, disease-resistant varieties of chick pea (*Cicer arietinum*) is an urgent need, since the supply of adequate protein still remains a problem in Pakistani diets especially for the young (A. Muhammed, University of Agriculture, Lyallpur). A. G. Kauser (University of Agriculture, Lyallpur) reported the successful development of reasonably high-yielding varieties with moderate resistance to wilt disease and high resistance to blight. C. M. Akhtar (Punjab Agricultural Research Institute, Lyallpur) reported varietal resistance to the major diseases of wheat, rice, sugarcane and cotton. Genetic resistance has not been observed against root rot of cotton, but biological control of this disease, by adding the antagonistic microorganism *Trichoderma viride*, along with organic substrate, has proved satisfactory but will need further field trials.

Soil salinity is the main problem in Pakistan especially where canal irrigation is practised, and at the moment the battle is being lost. Selection within hybrid rice populations for salt-tolerance appears feasible (M. Akbar, Nuclear Institute for Agriculture and Biology, Lyallpur) since crosses between salt-resistant varieties are more salt resistant than either of their parents and desirable progenies have been selected from F_4 populations. L. Bernstein (International Service Corps, New York) presented evidence that nutritional imbalance due to excess salt can often be corrected by foliar sprays of nutrients.

B. O. Eggum (Institute of Animal Science, Copenhagen) showed that up to 80% of the lysine in the wheat of some chappatis is made unavailable by cooking and that tannins, important constituents of tea, decrease the biological value of proteins. D. Boulter (University of Durham, UK) pointed

out the great potential for improvement in the yield of legumes; the alternative strategy of increasing the protein content of cereals would be a difficult task due to the yield/protein content "trade-off". That higher protein content can be achieved without serious yield penalty, however, was illustrated by the results of V. A. Johnson (USDA, Nebraska) and his colleagues, who have recently released a new productive high protein hard winter wheat variety derived from Atlas 66, with genetic potential for 2 percentage points higher grain protein content.

J. Dobereiner (Universidade Federal Rural do Rio de Janeiro) outlined the potential for nitrogen fixation of a number of tropical forage grasses and sorghum, maize and wheat; she reported that the *Spirillum* bacterium responsible is found inside the roots. Exploitation of this potential requires considerable work to identify the limiting environmental factors and develop agronomically feasible practices to overcome them. The difference between cultivars and even ecotypes of some species has been demonstrated and preference should be given to plant breeding; for example, among S_1 lines of maize, several could be selected with nitrogenase activity many times higher than that of the original cultivars.

J. K. Setlow (Brookhaven National Laboratory, USA) looking to the future, thought that genetic transformation with purified DNA would be of limited value in agriculture, but that the use of restriction enzymes had great potential, in generating sites for the insertion of specific genetic material. □

Thermodynamics in biological systems

from a Correspondent

An EMBO-sponsored Workshop on Thermodynamics in Biological Systems was held at the Villa Durazzo at Santa Margherita Ligure under the auspices of the University of Genoa on April 5-9, 1976.

THE contributions of thermodynamics in general, and calorimetry in particular, to the study of biochemical and biological processes formed the subject of the workshop.

S. N. Timasheff (Brandeis University) presented a thermodynamic analysis of the aggregation of tubulin which, in the presence of Mg^{2+} at low temperatures, yields cyclic polymers (molecular weight 3×10^6) composed of 26 subunits. Above 37 °C the aggregation to microtubules (molecular weight $\sim 10^6$) is favoured, particularly in the

presence of glycerol or sucrose. The thermodynamic and sedimentation data were confirmed by electron micrographs showing the different types of aggregates. A similar treatment, applied to the aggregation of sickle cell haemoglobin, was discussed by P. Ross (National Institutes of Health, Washington), on the basis of the polymerisation of critical nuclei, consisting of about 40 molecules, to helical structures.

With the aid of selective chemical modification of Trp 62 or Glu 36, J. Rupley (University of Arizona) demonstrated the involvement of these residues in the self-association of lysozyme. In the case of insulin, the three dimer-hexamer association characteristic of the crystal state is inconsistent with calorimetric, scattering and sedimentation studies in solution.

The thermodynamics of small molecule binding to biopolymers provided considerable interest. Thus H. J. Hinz (University of Regensburg) showed that whereas isoleucine tRNA synthetase showed a high affinity for isoleucine, compared with leucine or valine, yet ΔH and ΔC_p for the binding of all three amino acids are identical, suggesting COO^- and NH_3^+ groups as the interaction sites. M. N. Jones (University of Manchester) reported on the binding of sodium dodecyl sulphate to β -lactoglobulin which indicated two specific, high affinity sites ($\Delta H = -9.5 \text{ kJ mol}^{-1}$) and 22 non-specific sites ($\Delta H = -3.5 \text{ kJ mol}^{-1}$). In this context, but on a much more complex level, A. Beezer (Chelsea College) described applications of microcalorimetry to characterise interactions of microorganisms with drugs. Interpretation of such experiments are frequently complicated by specific interactions with certain buffer ions.

High precision heat capacity measurements on biopolymers over wide temperature ranges (5-500 K) continue to improve our understanding of the unfolding mechanisms. P. L. Privalov (Institute of Protein Research, Moscow) reported on methods of resolving $C_p(T)$ curves from various types of tRNA into several Gaussians which are found to correspond well with those derived from NMR measurements. The existence of "stable" intermediate states is therefore a possibility and the minimum domain size is estimated as about 100 residues. Comprehensive thermodynamic state diagrams, that is, free energies, enthalpies and entropies as functions of pH and temperature for both native and unfolded states, were presented by W. Pfeil (Zentralinstitut für Molekularbiologie, Berlin) for myoglobin, cytochrome c, haemoglobin, chymotrypsin and lysozyme. Such diagrams can now provide a thermodynamic base of re-

ference for "unfolded" proteins. J. Brandts (University of Massachusetts) discussed the unfolding of ribonuclease in terms of the possible intermediate states which are clearly indicated by kinetic measurements, although the thermodynamics are compatible with a two-state model, implying that all steps bar one are associated with zero enthalpy changes. This condition is met by the *cis-trans* isomerisation of proline which may therefore account for the rate-determining step, whereas conformational changes are associated with relaxation times of 1–100 ms. The folding/unfolding equilibria of all small proteins appear to be consistent with a rate-determining step corresponding to a *cis-trans* isomerisation.

A description of the dynamic nature of folded proteins presents many topical problems and both R. Lumry and A. Rosenberg (University of Minnesota) stressed the importance of high frequency (10^8 s^{-1}) conformational fluctuations to the catalytic functions of proteins. The existence of free volume fluctuations is consistent with the high diffusion rates of large molecules (such as acrylonitrile) in folded proteins and with the marked pressure dependence of proton exchange rates which are not observed with homopolypeptides or oxidised ribonuclease.

A thermodynamic characterisation of biochemical processes by itself is not always as interesting as an insight into molecular mechanisms, and this implicitly takes us into the realm of statistical thermodynamics. F. Franks (Unilever Research, Sharnbrook) discussed the combination of thermodynamic data and computational methods aimed at the derivation of a potential function for the hydrophobic interaction. This in turn allows the calculation of the radial distribution function and hence the thermodynamic properties associated with the interaction of apolar particles in water. In a complementary paper, E. Clementi (Istituto Donegani, Novara) outlined a combination of *ab initio* and computer simulation approaches to the study of amino acid hydration. □

Exploding black holes

from P. C. W. Davies

A symposium organised by R. Penrose and D. Sciama was held in Oxford on April 20 and 21, 1976.

BLACK holes are either a French, German or American idea, according to opinion. But if they explode, they are British. At an historic Oxford symposium

early in 1974, Stephen Hawking of the University of Cambridge, revealed an astonishing theoretical discovery—black holes are not black after all, but radiate with a characteristic blackbody (thermal equilibrium) spectrum, at a temperature which increases as the mass dwindles, ensuring the eventual demise of these objects in a sudden explosion.

Since then, the analysis of what is now referred to as the Hawking process has become a veritable industry in South East England, with no less than three major relativity groups (Cambridge, London and Oxford) actively studying the details and implications of the new discovery. Hawking's prediction itself was the result of applying quantum theory to black holes. The remarkable conclusion is that the violent distortion of space-time in their vicinity leads to the production of radiation with a definite temperature. The significance of this lies not so much in observation (the temperature of a solar mass black hole is a mere 10^{-8} K) but in establishing important theoretical connections hitherto barely suspected.

It had previously been noted by some relativists that black hole processes bear a superficial resemblance to thermodynamics. As long as the treatment remained classical the resemblance was only an analogy. The key step, which Hawking took, was to apply quantum mechanics. The result has been to provide an amazing underlying connection between quantum theory, general relativity and thermodynamics.

As experts in any of these three disciplines tend to be lamentably ignorant of the other two, it was particularly appropriate that this year's Oxford symposium entailed a programme of lectures and reviews by practitioners of each subject. The conference provided an opportunity for an unusual interplay of ideas, with the Hawking process as a central theme.

Hawking himself delivered some provocative and fascinating extensions of his original idea, by dealing with the conceptual and philosophical aspects of particle production effects. He and G. Gibbons (University of Cambridge) presented arguments in support of the proposal that event horizons cause radiation production under quite general circumstances. In particular, the claim that particle detectors in de Sitter space would also detect radiation seems to be a direct challenge to the conventional understanding of some fundamental aspects of relativity and quantum theory. This challenge was taken up by some of the other participants in fruitful but inconclusive exchanges. Controversy centred around Hawking's claim that much of physics

is observer-dependent. Two observers in different states of motion might encounter two entirely dissimilar histories of the Universe, he alleges. In particular, the traditional theoretical procedure of attempting to construct a covariant energy-momentum tensor which is independent of observer is misguided. Ironically, this procedure is precisely what several research groups have been trying to do, with a view to studying how quantum effects react back on the geometry of space-time. According to Hawking, the traditional energy-momentum tensor calculations are wrong. Indeed, they contain so-called vacuum polarisation terms, which he said he did not believe in.

It will take some time for the full implications of these intriguing new ideas to sink in. The more traditional approach was defended by Bryce DeWitt (at present at the University of Oxford). He described some of the conventional energy-momentum tensor calculations, and also presented an interesting model of a particle detector which appeared to conflict with Hawking's radical departure.

Much of the remainder of the conference was devoted to exposition about some of the peripheral aspects of the Hawking process. Some of the review talks were interesting and entertaining in their own right, particularly that of Donald Lyden-Bell (University of Cambridge) who presented a brilliant review of the thermodynamics of classical self-gravitating systems. Other speakers, such as Jergen Ehlers (Munich) who spoke about relativistic kinetic theory and statistical mechanics, and Bernard Schutz (Cardiff), who dealt with stability in relativity, clearly touched on subject matter which will eventually be relevant to the hoped-for unification of quantum field theory, thermodynamics and gravitation. But at this stage only a jumble of half-connected ideas has emerged. Other speakers were John Steward (Cambridge), Robert Thouless (Birmingham) and Oliver Penrose (Open University).

The cross-fertilisation between these subject areas is clearly of great importance in stimulating further research in this new subject. The conference organisers, Roger Penrose and Dennis Sciama, both gave thought-provoking general assessments, tying the strands of the separate topics together. It is hard to resist their own impression that much more lies beneath the surface; many disconnected ideas seem to be associated in some, as yet vague, but intriguing fashion. In this fast-moving field of research, it is probable that another Oxford symposium in the near future will provide still more fundamentally new results, which could well have implications far beyond the specialist topic of black holes. □

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The Zoo: 150 years of research

L. Harrison Matthews*

In the 150 years since its inception, the Zoological Society has developed from a collection of curiosities on which only observational research was carried out, to one of the world's most comprehensive collections of animal species, with two attached experimental research institutes. Dr Harrison Matthews traces the evolution of the Zoo from the time when the idea first occurred to Sir Stamford Raffles on a visit to the Jardin des Plantes in Paris.

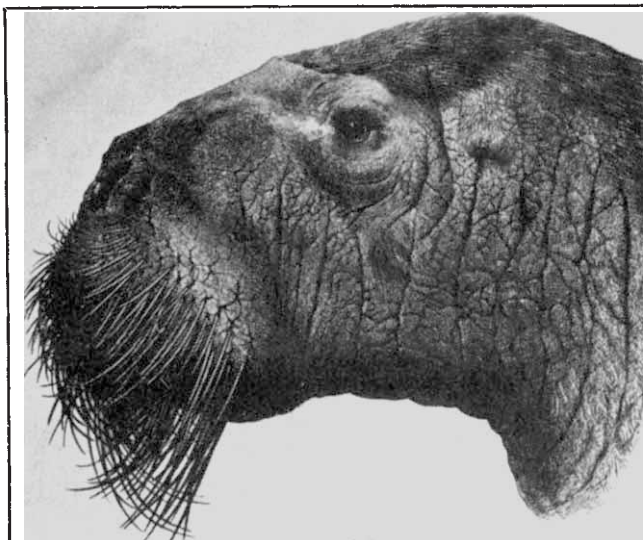
In the first quarter of the nineteenth century, towards the end of which the Zoological Society of London was founded, the science of zoology was emerging from the collecting of objects of natural history for the cabinets of the curious to become a discipline as scientific as botany and deserving the attention of people more serious than dilettanti. The works of Cuvier on the relationships of animals stimulated interest in the study of morphology among many naturalists who followed his researches on comparative anatomy and palaeontology. They broke away from the linear concept of classification built on the foundations laid by Linnaeus, who was the first to lecture in a university on zoology and botany as subjects in their own right instead of as adjuncts to medical knowledge. The immense numbers of hitherto unknown animals brought to the notice of European naturalists by the exploration and settlement of distant parts of the world gave descriptive zoology work enough to occupy many in describing and classifying the novelties.

Sir Stamford Raffles, who spent most of his short life in the service of the East India Company, and who was Lieutenant Governor first of Java and later of Sumatra, and was the founder of Singapore, gained a vast knowledge

of the plants and animals of the Far East, in particular the natural history of Java, Sumatra and the Malay Archipelago. In 1817, after leaving Java and before going to Sumatra, he was in Europe, and visited Paris where he was greatly impressed by the Jardin des Plantes. Later in the year he returned to England, where he was knighted and published his *History of Java*; but more important for the present subject he discussed with Sir Joseph Banks, then President of the Royal Society, a proposal inspired by what he had seen in Paris for the foundation of a zoological society in England, to be run on similar lines to the Jardin des Plantes.

Although Banks died in 1820 the proposal was kept alive by correspondence with Sir Humphry Davy and others after Raffles went to Sumatra in 1817, so that on his final return to England in 1824 he became chairman of a committee to consider the formation of the society. The committee's prospectus gave the objects of the society as "the introduction of new varieties, breeds, and races of animals for the purpose of domestication or for stocking our farm-yards, woods, pleasure-grounds, and wastes with the establishment of a general Zoological Collection, consisting of prepared specimens in the different classes and orders, so as to . . . point out the analogies between the animals already domesticated, and those which are similar in character upon

*The Old Rectory, Stansfield, Suffolk



A male bearded seal, *Trichechus rosamarus*. Taken from volumes of the *Transactions* of the Zoological Society.

which the first experiments may be made."

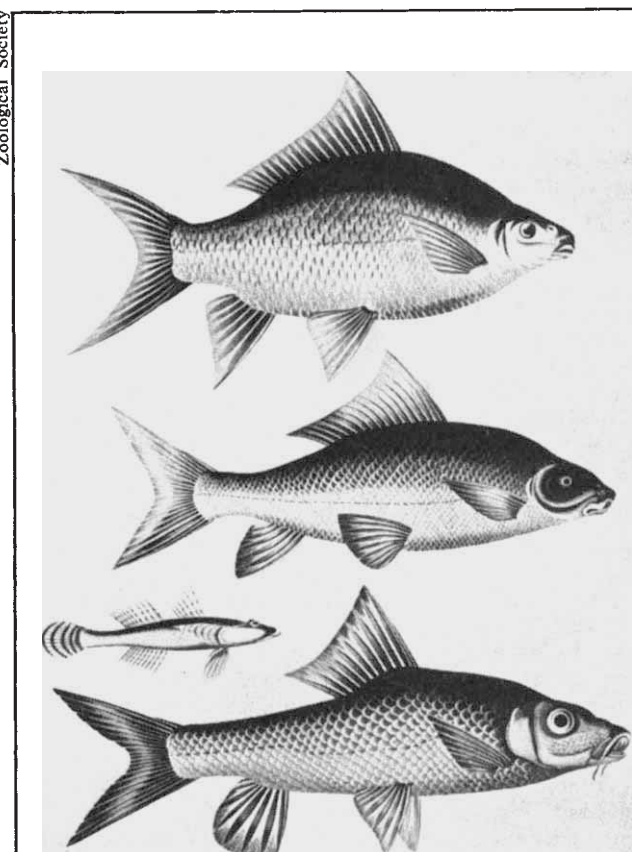
After long negotiations and the granting by the government of a site in Regent's Park the Zoological Society was founded in 1826 for the advancement of zoological knowledge and "the formation of a Collection of living Animals; a Museum of preserved Animals; and a Library connected with the Subject", with Raffles as president. Two months later Raffles died suddenly, and in the following year his widow gave the Society's museum his fine collection made in Sumatra; some of it went to the British Museum when the Society dispersed its museum in 1855. In 1829 the Society was incorporated by Royal Charter as "The Zoological Society of London" for the advancement of zoology and animal physiology, and the introduction of new and curious subjects of the animal kingdom—"animal physiology" had not then acquired its present meaning, but covered the feeding, breeding, and acclimatisation of animals. Raffles acknowledged that he himself was more interested in the scientific side whereas Sir Humphry Davy looked more "to the practical and immediate utility to the country gentlemen".

Raffles and Davy enlisted the help of the Aristocracy and Church in founding the Society so that the list of the first committee was full of "top brass" with a tail chiefly of amateur zoologists: in 1929 Chalmers Mitchell, then secretary, wrote that "from the beginning, the Society depended very slightly on the support of professional zoologists, and largely on persons of public importance—a tradition that it has always maintained." The Society's gardens were opened early in 1828, and the normal course of mortality soon began to supply specimens for examination and dissection by the anatomists, or for preservation in the Museum. The type specimens of newly described creatures were stuffed when they died and displayed in the cases; they were given to the British Museum when the collection was discarded but the rest of the contents was sold, some to the British Museum and some to various provincial museums.

Although a lecture was delivered to a general meeting of the Society in 1827 by Joshua Brookes, the proprietor of a private anatomy school, on the dissection of the body of an ostrich which had been given to the Society by George IV after it died at Windsor, scientific meetings did not start until 1830. At the first meeting for scientific business held in the autumn of that year the secretary, N. A. Vigors, an enthusiastic ornithologist, read a paper on the crested quails of America describing and naming two hitherto un-

known species. He was followed by Richard Owen who read a paper on the anatomy of an orang-utan that had died shortly after reaching the Society's collection and had been sent to him for dissection. These communications set the pattern followed by scientific meetings for nearly a century, concentrating for the greater part on systematics, faunas, the description of new species, and on morphology and anatomy.

In the 1830s a visiting veterinary surgeon treated sick animals and made occasional post-mortem examinations to find the cause of death in some of the animals that died, but the bodies of dead animals were generally given to such anatomists as were interested, for dissection and study in their own laboratories. It was not until 1865 that Professor T. H. Huxley spurred the Society into providing a dead-house and dissecting room, and to appointing a "prosector" in charge of them. This title was a misnomer, because a prosector is an assistant who prepares demonstration dissections for the lectures of a professor; the name was, however, retained for many years. The duties of the post combined those of pathologist and research anatomist, and the distribution of material to museums and academic workers who had asked for it to be supplied when available. For some sixty years the successive holders of the post gave greater attention to pathology or anatomy as they preferred—a large number of anatomical memoirs was produced by the earlier prosectors. In 1903 a pathologist as well as a prosector was appointed and, after a number of short term or part time workers had held the post, the Society in 1924 joined with the London School of Tropical Medicine and Hygiene, whose new buildings were not yet ready for occupation, in financing a Research Fellowship in Comparative Pathology, to the great advantage of the



From top to bottom: *Cyprinus abramioides*, *Varicorhinus babree*, *Gobus kurpah* and *Barbus mussallah*. Taken from volumes of the *Transactions* of the Zoological Society.

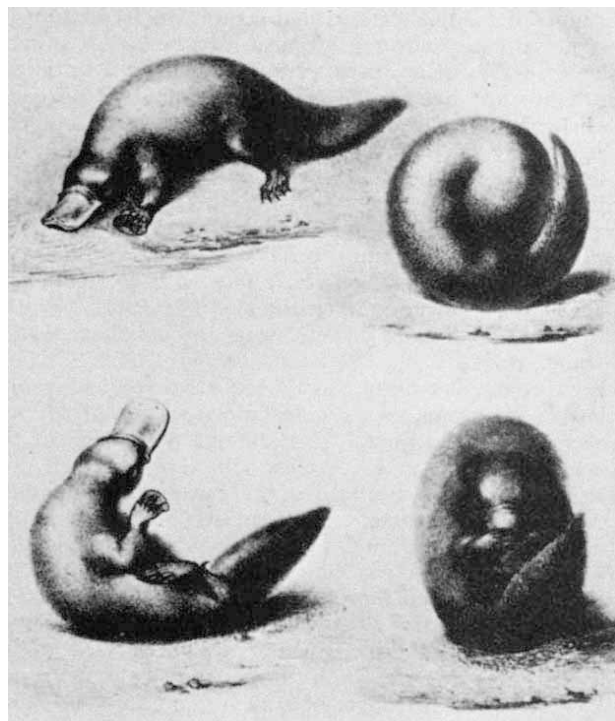
work on the Society's material. This arrangement lasted until 1928, when the Society appointed its own full-time pathologist, a post that continues today. In 1925 the post of prosecutor was abolished and replaced by a part-time research fellowship—though the title was revived later for a short time. Thus for nearly the first hundred years of the Society's existence the research carried out on its own premises was confined almost entirely to the descriptive anatomy of animals that died in the collection, some descriptive pathology, and the occasional description of new species from animals living in the gardens.

From the first, in 1830, the proceedings of the scientific meetings were published as a journal, which soon became the familiar "P.Z.S.", the *Proc. zool. Soc. Lond.* of reference lists. In its early years it was indeed a report of the proceedings, but as time went on it took the usual form of a scientific journal containing formal papers, some of which had been read in full at the scientific meetings, others only in summary or title, few of them based on work done in the gardens. The title was retained until 1965 when it was changed to the more appropriate *Journal of Zoology*—with a few sentimental sighs from some. In 1833 a second publication appeared, the *Transactions* of the Society, quarto in size to take papers that needed large illustrations; it was issued in parts at irregular intervals. The illustrations, especially those prepared in the last century, are superb, some of them very large folding plates, most beautifully lithographed and often hand coloured. Those of anatomical dissections and of bones, whether fossil or modern, are no less works of art than the illustrations of bright plumaged birds and of butterflies or other insects. Similar plates on a smaller scale were also often used in the *Proceedings*. The cost of producing these publications was great, and until recent decades was subsidised from the general funds of the Society, which thereby fulfilled one of its charter aims, of advancing zoology, in a very material way. With the fall in the value of money this is no longer possible, and the publications have to be financially self-supporting.

The publications were exchanged from their start for those of other learned societies all over the world so that part of their cost was recovered in kind to lay the foundation of the Society's magnificent zoological library. This is one of the largest and most comprehensive in the world, now housed in new premises with an excellent reading room and skilled staff appropriate to the importance and value of such a collection, to which all workers engaged in zoological research can have access.

From time to time the Society made grants towards the expenses of traveller-naturalists, generally with the stipulation that it should receive the living animals collected. Such collecting trips certainly advanced zoology in faunistic knowledge, but scarcely came into the category of academic research.

Apart from its publications the Society's support for research was thus mainly confined to observations on dead animals until the 1930s, when J. S. Huxley became Secretary. He was an early worker on animal behaviour following in the footsteps of Edmund Selous, who invented bird-watching in 1901; his well known paper on the courtship of the great crested grebe was published by the Society in 1914. In the few years before the outbreak of war in 1939 he himself studied animal behaviour on animals living in the Society's gardens, and helped others, among them James Fisher, in similar work. This trend towards the study of living animals is shown in the choice of subject by one of the early holders of the Research Fellowship, the present Secretary, who was appointed to the post as a young man shortly before Huxley joined the staff. Dr Zuckerman, as he then was, observed and analysed the behaviour of the colony of *Hamadryas* baboons that made "Monkey Hill" one of the sights of London, approaching the subject "from the deterministic



Duckbilled Platypus, *Ornithorhynchus paradoxus*. Taken from volumes of the *Transactions* of the Zoological Society.

point of view of the physiologist", although he is described as "Anatomist to the Society" on the title page of the book in which he records and discussed his researches. This was what would now be called a seminal work, though its conclusions have been modified in the light of work on animals in less unnatural surroundings and in populations of more normal sex ratio.

The outbreak of war in 1939 put an end to this new approach to research: by 1945 a long list of dilapidations had accumulated, so that neglected repairs, the restoration of bomb-damage, and replenishing the collections took most of the Society's current resources until the 1950s when the present Secretary, now Lord Zuckerman, was appointed. The old sanatorium, and the prosectorium which combined dissecting-room with pathology laboratory, were quite inadequate for any serious work, though they had served well enough in their day. A new and well equipped animal hospital, and modern pathology laboratories were built, a full time veterinary officer and a new pathologist were appointed with a supporting staff of qualified technicians. When vacancies occurred new curators were appointed to the various departments, so that they are now all academically qualified zoologists. A steady stream of research publications has flowed from the work of these officers and from visiting workers in their departments.

The most impressive developments in research, however, are the establishment of two research institutes in the Society's gardens, with splendidly equipped modern laboratories, animal rooms, and ancillary services. The Nuffield Institute of Comparative Medicine, and the Wellcome Institute of Comparative Physiology owe their conception to the foresight of Lord Zuckerman, and their realisation to his genius for fund-raising and to the generosity of several philanthropic foundations and trusts. The work undertaken by the institutes differs fundamentally from the researches formerly sponsored by the Society in being experimental

and not solely observational. The latter were necessarily restricted to work that does not need a Home Office licence under the Cruelty to Animals Act, because considerations of public relations and sentiment, in addition to the Act itself, preclude any experimental work on animals exhibited in the Society's collection: the institutes, on the other hand, are licensed. The latest available reports on the work of the Institutes show the wide range of subjects covered, many of them of immediate value in preserving the health and well-being of the collections. In the Nuffield Institute of Comparative Medicine the departments for pathology, infectious diseases, biochemistry, and radiology have investigated numerous problems arising in the collections, as well as more general topics with application to human as well as veterinary medicine. The Radiology Department also maintains the growing and much consulted Wellcome Animal X-ray Museum. The Wellcome Institute has been concerned mainly with the physiology of reproduction in mammals. An investigation of special interest has been carried out on the reproductive physiology of the hystricomorph rodents, some of which were collected during visits to South America for the purpose by members of the staff. Several of the species have bred successfully in the Institute. Other studies include work on the viability of spermatozoa, on the possibility of artificially inseminating African elephants, and reproduction in the Mustelidae. The Institute also contains a growing reference collection of reproductive and endocrine organs.

The Institutes give hospitality to a steady flow of visiting workers, and send members of staff abroad for field work

or to visit other institutions, with some of which they have special affiliations. The activity of the Institutes is illustrated by the long list of scientific papers published each year by members of staff and visitors, in the *Journal of Zoology* and the publications of other learned societies.

Since 1959 the Society has held several symposia every year on special topics to bring together workers engaged in research on different aspects of any branch of zoology. The papers and discussions are printed in a special series of Symposia publications which has a wide circulation. The *International Zoo Yearbook*, published annually since 1960 is addressed mainly to those concerned with the housing and husbandry of animals in captivity, but it does contain accounts of research pertinent to those subjects.

Finally, an important publication of long standing must be mentioned. The *Zoological Record*, issued annually since 1864, is an essential tool to all engaged in research on zoology; it is an international bibliography listing all papers published in all branches of zoology for each year, with author, subject, and systematic indexes. The enormous growth in the literature during the past twenty years is reflected in the size of the annual volume, now prepared by a whole-time staff of specialist recorders.

The shades of Raffles and Humphry Davy, could they glimpse the developments at Regent's Park as they stroll through the groves of the Elysian Fields, could surely be surprised to see what they had started, and would be gratified to know that their ideas for the advancement of zoology are being so energetically realised in the researches made possible by their Society.

articles

Isotopic tree thermometers

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Evidence is summarised here that trees store a record of atmospheric temperature in their rings. In each ring, the ratios of the stable isotopes of hydrogen and oxygen vary with the air temperature prevailing when the ring was formed. We have shown that the temperature records in three modern trees seem to follow the local mercury thermometer records, and have found that a Japanese cedar indicates a temperature fall of $\sim 1.5^\circ\text{C}$ in the past 1,800 yr.

LONG TERM isotope changes in precipitation, caused by changes in climatic temperatures, are well documented in polar ice caps—the heavier of the stable isotopes is depleted in ice laid down in the ice age by comparison with present-day ice. In 1970 we extended this concept to trees, suggesting that they are also thermometers, and undertook measurements to test the idea. In trees which use atmospheric precipitation to manufacture their rings, isotope variations in the rings should be climate indicators because the isotope composition in the rain and CO_2 varies with temperature.

Many tree ring sequences can be counted with an accuracy of about one year. Those which are not yet tied to modern sequences by overlapping ring patterns (said to be "floating"), can be dated in favourable cases with an accuracy of about 30 yr, by radiocarbon dating, depending on the age and on the number of radiocarbon measurements which are made. The isotopic compositions of atmospheric carbon dioxide, rain and snow have been shown to vary strongly with atmospheric temperature^{1,2}. A non-negligible temperature-dependent isotope fractionation in the formation of wood has been computed assuming thermodynamic equilibrium but is small by comparison with that expected in rainwater³. Therefore, the tree ring emerging from the outer living layer of sapwood should record the average temperature of the atmosphere at that time, except for possible isotope exchange between the heartwood and

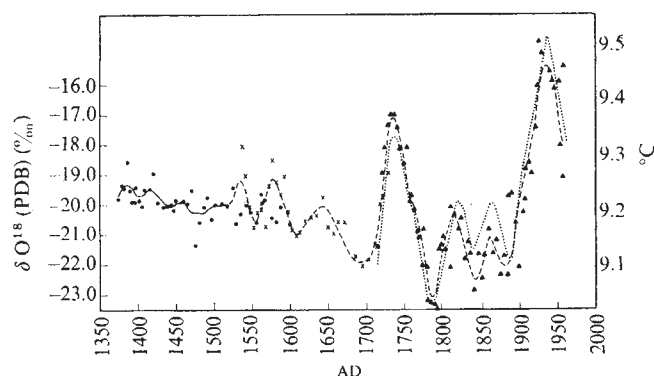


Fig. 1 ^{18}O ratio in German Oak, *Quercus petraea* (Spessart Mountains), compared with the annual average temperature of England (—). Thirty-year running averages of each isotope ratio are plotted against tree ring years. Five-sample (or ~ 30 yr) running average for oak from Marburg, Germany; $\bullet$, 4.7-yr sample; $\blacktriangle$, Seven-sample (~ 30 yr) running average for Spessart oak No. 1; $\times$ five-sample (~ 30 yr) running average for Spessart oak No. 2. The temperature record of England is shown here for comparison because it extends much further back than do the next most lengthy records, namely those for Basel and Geneva. The agreement with these latter temperature records is equally good, however. PDB, Pee Dee Belemnite, a fossil shell used as a standard; — — —, smoothed by eye.

water. The matter of isotope exchange between intact heartwood and water does not seem to be a serious threat since it has been shown, at least in species having tight rings, that the sap does not interact with the heartwood. Huber has shown, using biological dyes in many tree species, that water conduction remains limited to the outermost annual ring⁴. In addition, radiocarbon injected into or ingested by a living tree does not exchange with neighbouring rings^{5,6}. Furthermore, we have observed that when *Sequoia gigantea* wood was soaked for 37 d in an air atmosphere saturated at 100% humidity, and previously labelled to $\delta\text{D}_{(\text{SMOW})} = 1,170\text{‰}$, no deuterium exchange was detected even though water saturation was optically evident. This is reasonable because wood is a most remarkable polymer, containing very large molecules, largely cross linked internally and to each other with hydrogen bonds. Furthermore, the bonds of cellulose and lignin are very strong, by comparison with almost any other molecular bonds, so that isotope exchange at room temperature with water or dissolved CO_2 becomes very improbable.

Temperature records

The trees most useful for testing this method of determining temperature histories and making calibrations are European trees since only Europe has temperature records extending back three centuries⁷ (for England) and more than two centuries (for Basel and Geneva).

To demonstrate the effect experimentally, we needed to measure modern trees whose rings have been correctly counted and to obtain lengthy mercury temperature records from places near where they grew, preferably from the same altitude. The oldest temperature records in the World Weather Records are for Basel and Geneva, at about 300 m. We therefore obtained from the laboratory of B. Huber in Munich (the only tree ring laboratory in Europe when we started this work) a German oak, grown in middle Germany at an altitude of about 300 m, which had been correlated with the fiducial tree ring sequential pattern developed by Huber and his colleagues⁸. Later we obtained samples of similarly calibrated trees from his students, B.B. and D. Eckstein.

So far, we have measured isotope ratios in three modern trees of three different species and have compared them with local temperature records from mercury thermometers; we have also

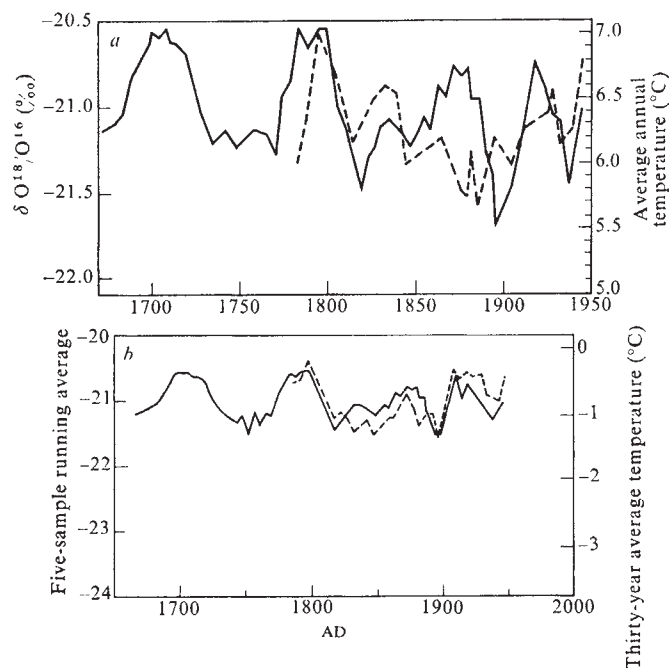


Fig. 2 ^{18}O ratio (—) for Bavarian fir, *Abies alba*, compared with: *a*, annual average temperature; *b*, average of January, February and March temperature for Höhen Peissenberg, Bavaria, at the same altitude of about 1,000 m above sea level, but about 150 miles south-west of the Bavarian Forest where the fir grew. This is mountainous country so that local differences in temperature may be expected. Temperatures for January, February and March seem to fit as well or better in all three modern trees analysed so far. This might perhaps be explained by the fact that the trees we have analysed up to now have grown where winter snow collects and melt water freezes into the ground. When the ground thaws, sap begins to flow (for example, the voluminous flow of maple sap and turpentine sap in early spring) and growth begins (for example, the fast rate at which pines shoot their candles in early spring). In the past three centuries, judging from mercury thermometer records, the major climatic change has been in temperatures during winter months. Temperatures in summer months have remained relatively constant since thermometer records began.

studied two old trees of two different species and compared the isotope ratios with temperature records deduced from surrogate evidence such as lateness of cherry tree bloomings, number of snow days per year, and number of days per year when lakes were frozen. We found significant correlations.

In Fig. 1 we compare ^{18}O measurements for a German oak, *Quercus petraea*, the rings of which were counted by Huber and Siebenlist⁸, with English temperature records⁹⁻¹¹ back to

the time of the invention of the thermometer in the late seventeenth century. The temperature coefficients have been evaluated by least squares analysis^{12,13}. Figure 2 shows the ^{18}O data for a Bavarian fir, *Abies alba*, the rings of which were counted by Becker and Siebenlist¹⁴; they are compared with temperature records made near where the tree grew⁷. Similar results have been obtained with deuterium measurements (Fig. 3) for the oak.

General applicability

Since the method seems to have worked for European oak and fir, we felt that it would probably work elsewhere. Direct measurements of the isotopic composition of atmospheric

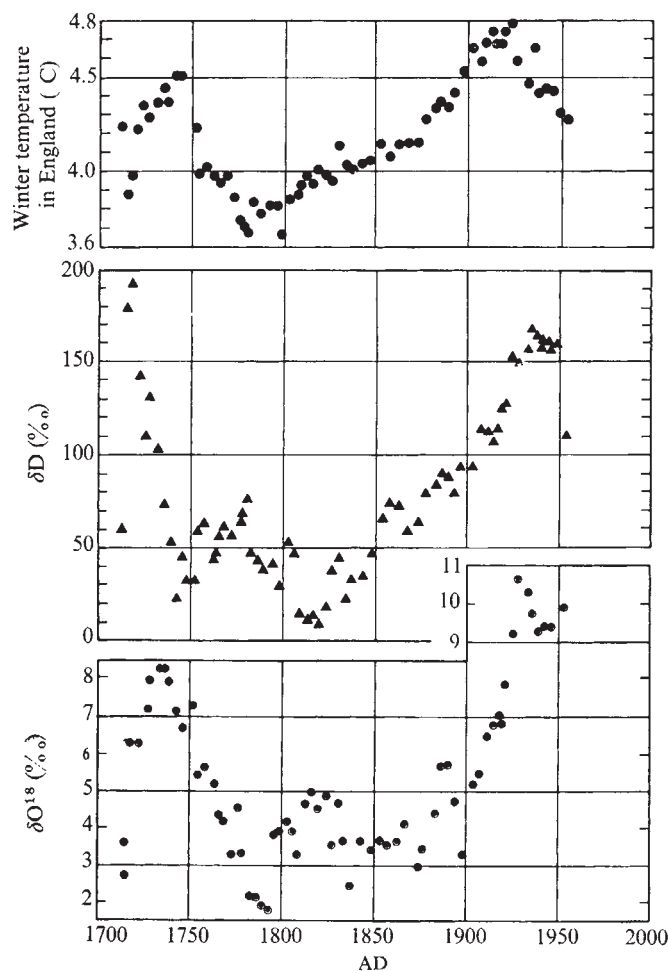


Fig. 3 Deuterium and oxygen ratios in German oak compared with English winter temperatures (30-yr running averages, see ref. 11). The procedure we used was to sample with a milling tool a number of tree rings, obtaining about 5 mg per sample; heat with HgCl_2 to produce CO_2 and HCl (ref. 22); remove the HCl with quinoline; and measure the ^{18}O abundance in a mass spectrometer. For measurement of the deuterium, we burnt the wood, reacted the water with metallic uranium to produce hydrogen gas and measured its D abundance in a hydrogen mass spectrometer (negative ratios).

moisture (as rain and snow) and indirectly of carbon dioxide, the ^{18}O of which is probably in isotopic equilibrium with atmospheric moisture¹⁵, have been made and are available in the publications of the International Atomic Energy Agency (IAEA)³. Figure 4 shows the ^{18}O and D correlation with temperature for Austrian stations for the past 15 yr or so. The isotope composition of precipitation varies similarly with

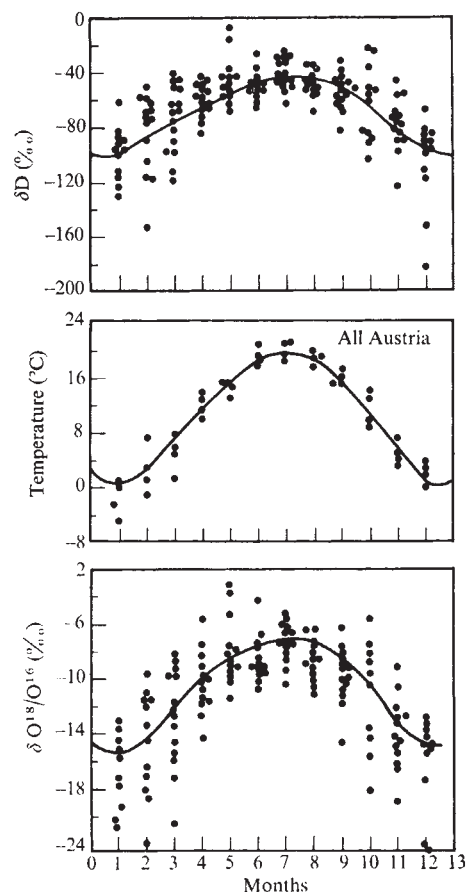
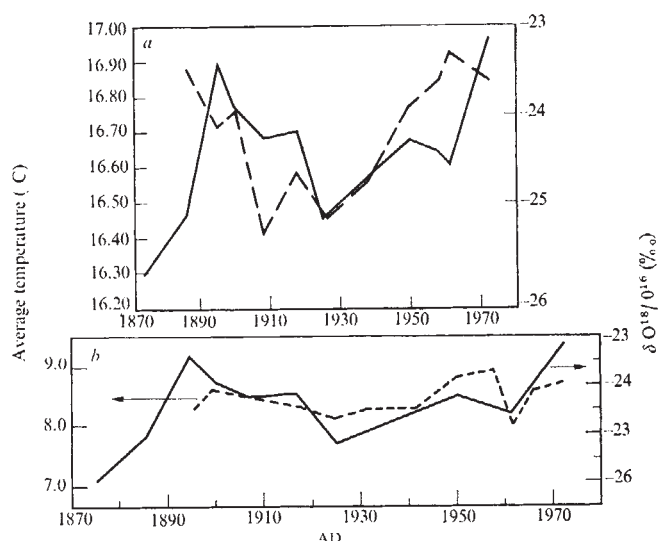


Fig. 4 Deuterium and ^{18}O isotope measurements in Austrian precipitation (Vienna 1961–71, Petzenkirchen 1966–71, Apetlon 1966–69, Podersdorf 1966–68) compared with average monthly temperature (Vienna 1961–64) taken from ref. 2. We have also plotted the IAEA data from these years for Stuttgart and many other places and find similar temperature dependences. When the climate deteriorates or improves, presumably the curve of annual dependence moves down or up on the isotope axis; the IAEA data have been measured for only about 20 yr, so long term changes have not yet been demonstrated.

Fig. 5 ^{18}O (—) in modern *Cryptomeria japonica* compared with: a, average annual; b, January, February and March air temperatures measured at Miyazaki, Japan¹⁰. The *Cryptomeria* grew at an altitude of 1,350 m, whereas Miyazaki is at sea level, about 150 miles north-east of Yaku Island. Averages over about 8 yr are shown.



temperature in many other places, as may be seen by plotting the IAEA isotope data against air temperature².

Old Europe and Japan

Thus we have had the temerity to measure German oak rings which grew before thermometers were available⁶. In Fig. 1 we show warm intervals at AD 1530, 1580 and 1650 and cold periods at about AD 1700 and 1800, in agreement with Bergthorsson's deductions of climate variations in Iceland¹⁶, and in agreement with the historical evidence of severe climate

allowing us to calibrate the isotope changes in terms of temperature differences.

Measurements of the isotopic composition of atmospheric moisture are compared, in Fig. 6 *a* and *b*, with temperatures deduced from surrogate evidence obtained from old diaries for Japan¹⁹ and for China²⁰. Figure 6*a* raises several questions. Are the tree ring ages correct in this tree? Is the surrogate evidence giving rise to the Yamamoto and Chu comparison temperatures and to the Norwegian snow line data either pertinent or correct? It may be possible to find other evidence for the temperature changes indicated by the isotope variations

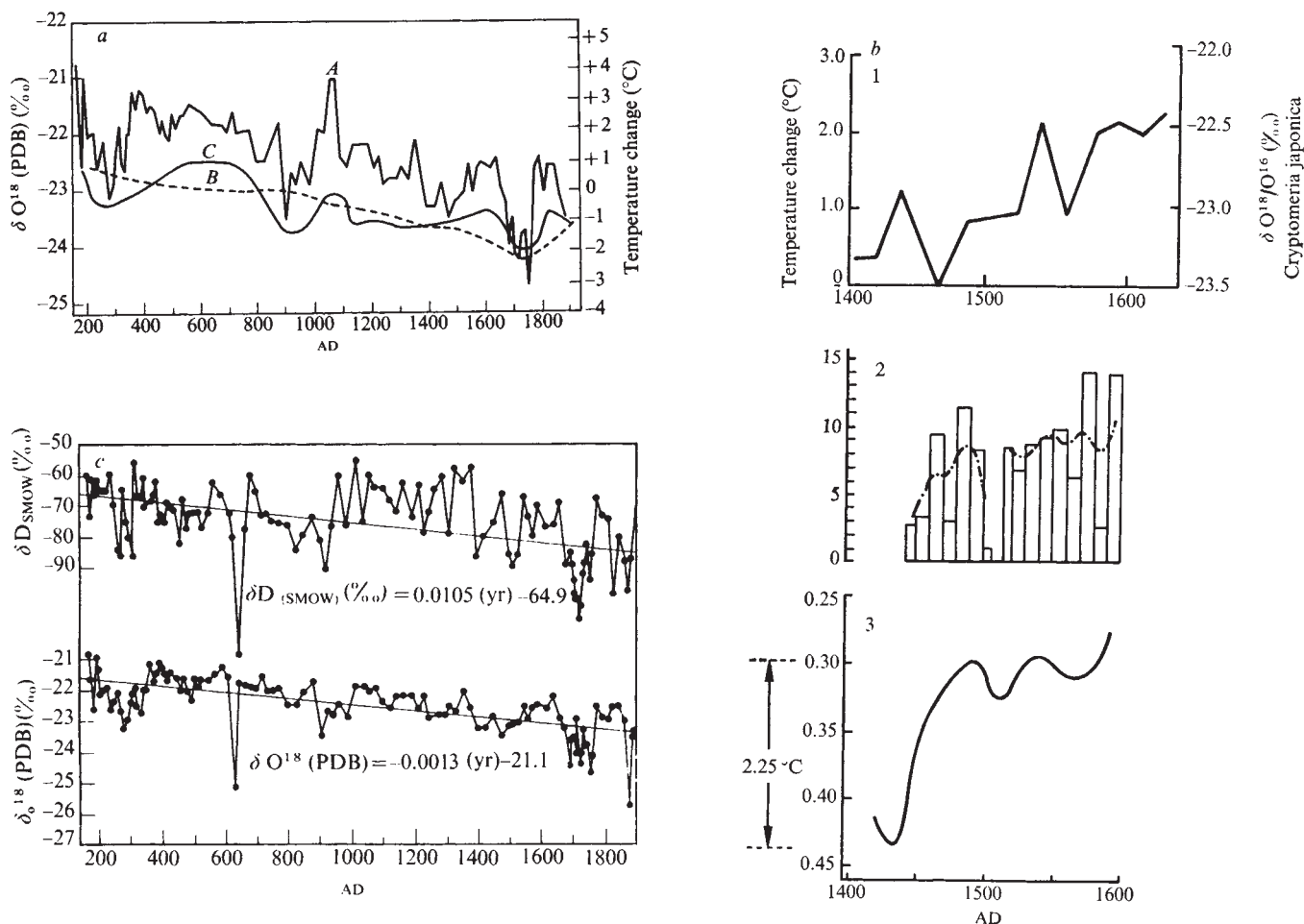


Fig. 6 ^{18}O ratio (*a*) in historic *Cryptomeria japonica*: *a*, compared with Chinese air temperatures (*b*) and Norwegian snow line (*c*)¹⁹; *b*, compared with Japanese air temperatures¹⁸. Averages over about 8 yr are shown. *b*: 1, oxygen data against temperatures obtained from cherry tree blooms; 2, change of the first freezing date of Lake Suwa; 3, change of number of snow days a year, calibrated by comparison with modern changes and modern temperatures. *c*, D/H ratio and $^{18}\text{O}/^{16}\text{O}$ ratio against time, the least squares fits against time, and the least squares fit to the dependence of δD on $\delta^{18}\text{O}$ with a slope of 8.2.

deterioration in the First and Second Little Ice Ages in Europe.

Further, we have measured an old Japanese cedar, *Cryptomeria japonica* or Yaku-Sugi (the ^{14}C in the rings of which were measured by Kigoshi¹⁷), for both hydrogen and oxygen isotope ratios. Kigoshi counted the rings of this tree and verified the count by making 50 radiocarbon datings on them. Ten dates were made on the oldest part of the tree, each to an accuracy of ± 100 yr, for the years counted as AD 137–537. The accuracy of his count in the oldest part is thus substantiated to an accuracy of $\pm 100/\sqrt{10}$ or ± 30 yr, by the radiocarbon evidence. In Fig. 5 we have compared the modern part of the cedar with temperatures¹⁸ measured at Miyazaki, a nearby weather station. Although Miyazaki is at sea level, our *Cryptomeria* grew at a higher altitude, about 5,000 feet. In spite of climatic differences which might be expected to arise from difference in altitude, there seems to be significant agreement,

in the cedar and thus indirectly check the method further.

The fact that

$$\delta\text{D} = 8.2\delta^{18}\text{O} + \text{constant}$$

for the cedar, showing a slope consistent with that for worldwide rainwater within experimental error^{12,21}, indicates that the tree is storing old rainwater. Furthermore, it supports the hypothesis that the CO_2 and H_2O are in isotopic equilibrium in the atmosphere.

The absolute values of the deuterium ratios, about -70 SMOW, are about the same as in rainwater. The absolute values of the oxygen ratios, around $+20$ SMOW, are halfway between that for rain (about -7 SMOW), and that for atmospheric CO_2 (about $+40$ SMOW). This is to be expected for water vapour in equilibrium with atmospheric carbon dioxide and keeping in mind that carbon dioxide absorbed by the leaves exchanges its oxygen with sap.

We have made Fourier transforms of the data in Fig. 5c to deduce the power spectrum of periodicities. The results are shown in Fig. 7a and b, and in Table 1. The same periods are found in both D/H and in $^{18}\text{O}/^{16}\text{O}$ data, within experimental error, as obtained from the widths of the peaks in the power spectra.

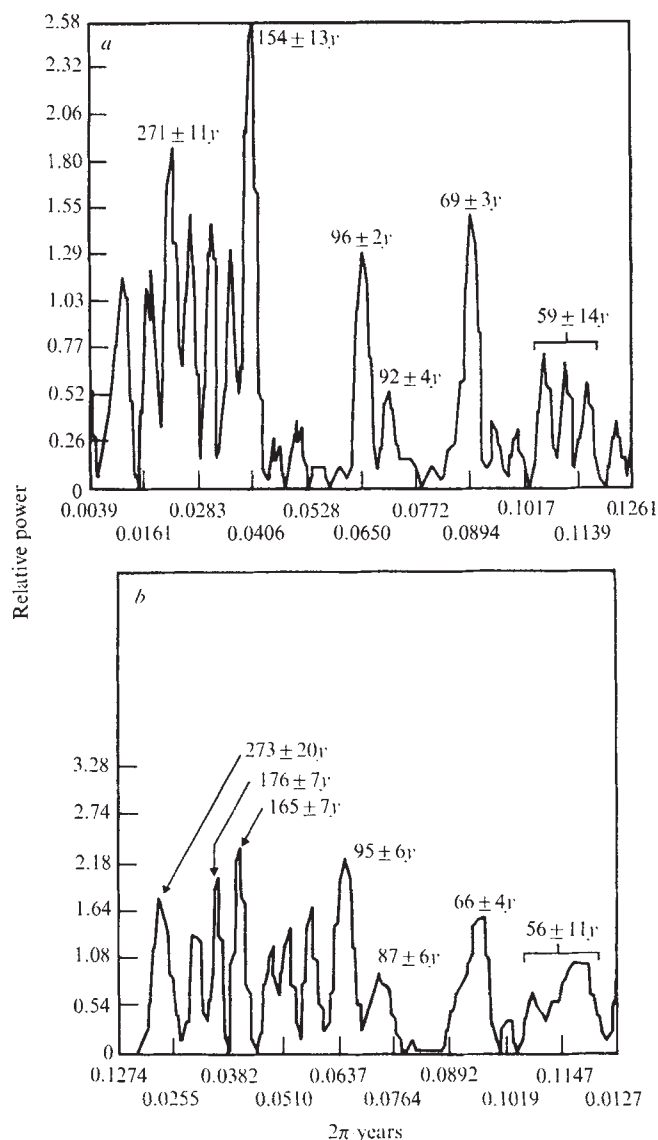


Fig. 7 Power spectra obtained from Fourier transforms of the data in Fig. 6c. The indicated periods are not sensitive to various ways of cutting off the ends of the data. a, Fourier transform of $\delta^{18}\text{O}$, average slope subtracted; b, Fourier transform of δD , average slope subtracted.

Our samples represented approximately 5 yr each, so we cannot expect to find evidence for periods of less than about 40 yr in these data. As the sets of data span only about 1,800 yr, we cannot expect to find evidence for periods of more than about 250 yr. Both data sets show a long term decline of about 1.6°C in average temperature over the past 1,800 yr. This is significant, especially considering that there was a decline of about 10°C in the last ice age. In making the Fourier transforms we have used the deviations of the isotope ratios from the long term slopes—that is, we have corrected for the slope. One may ask whether the periods of about 58 yr and about 68 yr are really different from each other, and likewise for the periods of about 90 yr and 95 yr. The peaks in Fig. 7 suggest that they are. We need to measure more trees to find out. We varied the methods of cutting off the data at the end points to confirm that the period of 272 yr is real. Also we fed artificial periodicities

Table 1 Periods and their respective powers found in Fourier transforms of oxygen and hydrogen stable isotope data in Japanese Cedar AD 140–1950

$\delta^{18}\text{O}$		δD	
Period (yr)	Relative power	Period (yr)	Relative power
59 ± 14	0.67	56 ± 11	1.00
69 ± 3	1.50	66 ± 4	1.64
92 ± 2	0.52	87 ± 3	1.00
96 ± 2	1.29	95 ± 2	2.18
154 ± 6	2.58	153 ± 7	2.18
174 ± 7	1.29	174 ± 8	2.00
202 ± 7	1.45	205 ± 9	1.70
271 ± 11	1.80	273 ± 20	1.64

of 50 and 55 yr and of 90 and 95 yr into the Fourier transform and found them separable.

Future research

It seems wise to measure more modern ring sequences and compare them with local mercury thermometer records in order to establish the tree thermometer phenomenon beyond question. And, of course, there are experiments to be done growing woody material in conditions of controlled temperature and isotopic ratios in atmosphere and water. We plan also to study old tree ring sequences. We have such sequences in our laboratory, including sequoias, firs, cedars, and pines provided by B. Becker, Dieter Eckstein and other experts on tree ring counting.

When we have measured isotope ratios in each ring of a lengthy sequence (as opposed to making measurements of five rings at a time) we shall explore the Fourier transforms of the data for evidence of the sunspot cycle of about 21 yr and other periodicities of less than 50 yr and for sum and difference frequencies. When we have determined whether there are shorter periodicities, and substantiated the longer periodicities listed in Table 1, we plan to put them in the phase relationships indicated by the trees and predict the trends of climate to be expected for the next twenty years or so.

The exciting possibility of studying trees felled by the advancing ice sheet at the Two Creeks site in Wisconsin 12,000 yr ago is an obvious application of this technology. It seems likely that if the method is sound we can establish the history of climate back many millennia, as far back, in fact, as there are tree remnants to be found.

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Stratospheric ozone as viewed from the Chappuis band

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Stratospheric total ozone values have been obtained for the period 1912–50 from analysis of Smithsonian data. Naturally caused variations of 25% or more are common over time scales ranging from months to decades. We suggest that the ozone layer acts as a shutter on the incoming solar energy, providing one of the long sought trigger mechanisms between solar activity and climatic change. The present state of knowledge of ozone climatology is not sufficient to support popular speculations about man's effects on the ozone layer.

Much speculation has been published about the naturally occurring ozone in the Earth's stratosphere. The controversy has centred around theoretical attempts to demonstrate that certain of man's activities such as the flight of SSTs or the production and release of fluorocarbons will reduce the mean amount of naturally occurring ozone and lead to unwanted biological effects¹. These theoretical speculations generally predict a total depletion of nearly 7%. Because of its absorption bands in the ultraviolet, visible and infrared parts of the spectrum, ozone is also an important climatological parameter. Thus it is particularly interesting to examine the history of natural variations in ozone amounts over a time span of several decades.

We are trying to determine historical baselines for atmospheric constituents using data obtained by the Smithsonian Institution's Astrophysical Observatory (APO) in their solar constant observing programme from 1902 to 1961. As a normal part of their observing procedure, they obtained spectrophotometric tracings of the direct solar radiation reaching the ground over the spectral range 0.35–2.2 μm . We have previously analysed the published portion of these data for changes in atmospheric transmission caused by suspended aerosols². We have now extended our analysis of these data to determine values for total stratospheric ozone above high altitude stations in southern California from 1912–1950 and northern Chile from 1918–1948.

In the past, virtually all long term monitoring programmes for total ozone have used Dobson spectrophotometers or other instruments such as filter photometers. These instruments operate in the ultraviolet part of the spectrum to observe the strong ozone absorption in the Huggins bands (0.30–0.35 μm). The APO transmission measurements included the Chappuis band (0.5–0.7 μm) and therefore offer both an extension of the baseline in time and place, and a valuable check on the Dobson ozone amounts determined with this different band. The measured transmission in the Chappuis band has been reduced by ozone, Rayleigh scattering, and aerosol scattering. We used

the transmission data outside the ozone band to calculate the Rayleigh and aerosol components. Absorption coefficients from Vigroux³ were then used to calculate the total ozone.

The Chappuis band has generally not been used for ozone because it is a very weak feature. The APO data are, however, of sufficient quality that we were able to model the Rayleigh and aerosol contributions so that the resulting daily probable error in ozone amounts was only 7%. An advantage of the Chappuis band is that its absorption coefficient is unaffected by temperature³—a property not shared by the ultraviolet bands.

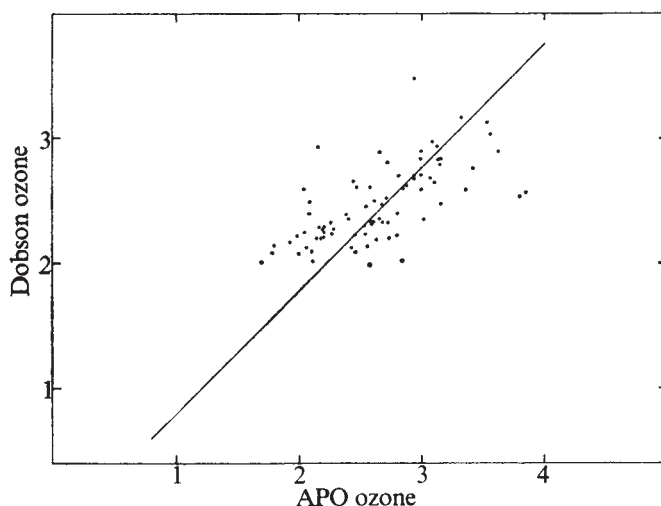


Fig. 1 Correlation between ozone amounts determined from 78 d of observation at Table Mountain, California with both a Dobson spectrometer and an APO spectrophotometer. Ozone concentrations here and in subsequent Figs are measured in mmHg (NTP).

A comparison between Dobson values and our values is possible from 78 d of nearly simultaneous APO and Dobson observations made at Table Mountain, California from August, 1928 to May, 1929 (Fig. 1). A simple linear regression analysis yields a correlation coefficient of 0.7 and a slope of 0.98 between the two data sets. The lack of perfect agreement is attributed to several causes. First, the APO observations were carried out a few hours earlier on each day than the Dobson work. Second, the ozone absorption coefficients are not precisely known; inaccuracies of up to 10% may still exist⁴. Third, the calculated error for values derived from the daily APO measurements is 0.23 mm s.d. in the APO–Dobson comparison. It is for this last reason that we present here monthly mean values of ozone, where the calculated error is reduced typically to ~0.10 mm.

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Figures 2 and 3 show monthly mean and 12-month running mean total ozone amounts for southern California and northern Chile, expressed in terms of the equivalent height of a vertical column of pure ozone at NTP. Each point before 1930 represents the mean of ~ 6 d. Observations were made at Mt Wilson only during the summer months. Only ~ 50 values per site were published for the period 1940–1950; thus each point in that period represents typically 1 or 2 d compared to typically 6 d for the earlier period.

It is apparent from Figs 2 and 3 that at both sites total ozone amounts commonly show variations of as much as 20–30% on time scales ranging from months to decades. Comparison with other work⁵ shows that such variations are not at all unusual in Dobson spectrophotometry. We also present 12-month running means (dashed line) for comparison with the highly smoothed data usually found in the literature. Even here large variations are found that are comparable to those recently reported by other workers. For instance, Angell and Korshover⁶ found changes of up to 8% over 3 yr, and Komhyr *et al.*⁷ found changes from 2–10% per decade for the period 1960–70. We find that such changes occurred throughout the forty-year APO record analysed here.

Because the large natural variations in the ozone layer are not uncommon, consideration of the amount of incident solar energy absorbed by the Chappuis band suggests a new

On the other hand, because of its location near the peak of the solar energy spectrum, the Chappuis band absorbs on average $\sim 2\%$ of the total solar flux at midlatitudes¹¹. Thus, for example, if solar variations and stratospheric transport combine to produce an increase in stratospheric ozone of 25% over a given mid-latitude station, the total solar energy reaching the troposphere above that station would be decreased by $\sim 0.5\%$, an amount of importance to both weather and climate. Thus ozone can serve as a stratospheric shutter operated by ultraviolet rays but modulating the energy flux in the visible region. Because of the longer slant path lengths and larger amounts of ozone, the effect is much larger in the polar regions. Ozone climatology is further complicated by the absorption band at $9.6\ \mu\text{m}$ which makes an important contribution to the greenhouse effect^{12,13}.

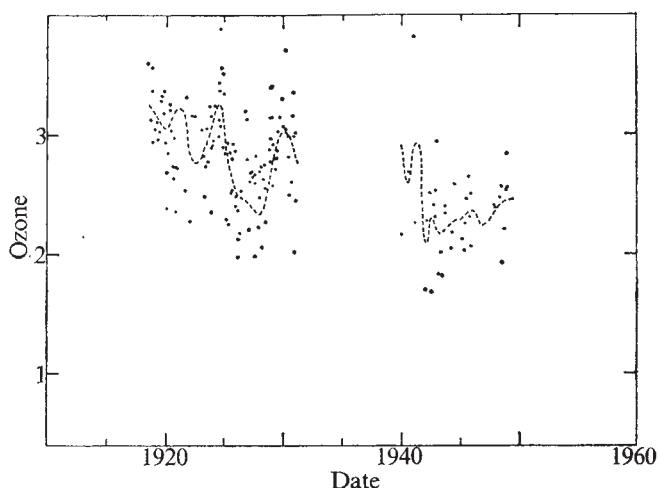


Fig. 3 Monthly mean total ozone for northern Chile sites Calama (1918–20) and adjacent Mt Montezuma (1920–30 and 1940–48). The dashed line represents twelve month running means. Typical probable error is 0.10 mm.

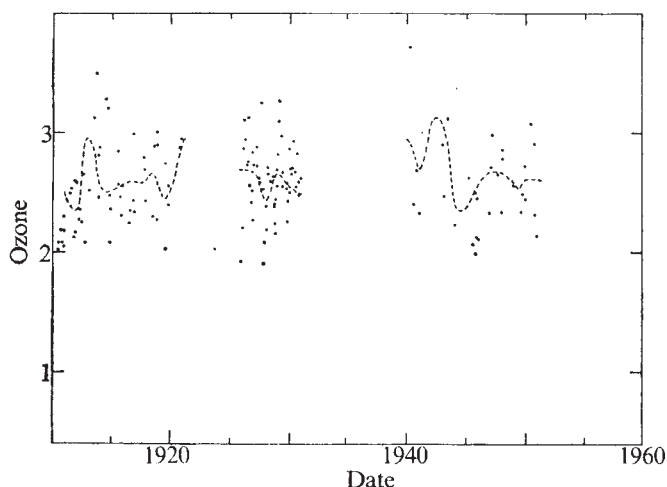


Fig. 2 Monthly mean values of total ozone for the southern California sites Mt Wilson (1912–20) and Table Mountain (1925–30 and 1940–50). The dashed line represents twelve month running means. Typical probable error is 0.10 mm.

perspective on the long sought link or 'trigger mechanism' between solar activity and the Earth's weather and climate⁸. Stratospheric ozone is created photochemically by solar radiation in the wavelength region from $\sim 1,800$ to $2,100\ \text{\AA}$ (ref. 9). Although the solar energy output shows substantial variations in this region and at shorter wavelengths¹⁰, this total energy change is such a small fraction of the average solar output that it is generally considered to fall far short of meteorological significance. Also, photons in this wavelength region are absorbed in the stratosphere, requiring that any tropospheric effects should be indirect⁸. Since the ultraviolet Hartley and Huggins bands are saturated and Rayleigh and aerosol scattering are very large in the ultraviolet, the net atmospheric transmission of radiation in the near ultraviolet is not strongly affected by variations in stratospheric ozone.

The possibility that man might inadvertently modify the ozone level is a matter of much concern. The presence of large variations in ozone amounts makes it clear that we must really think in terms of a natural range in ozone amounts, rather than a single level. Considering the magnitude of the spatial and temporal variations involved, there exists only a relative handful of measurements of past ozone levels, and it is not now possible to say what might constitute an optimum ozone level at a particular time and place, or if such a level can even be defined. Finally, we note that because of the presence of natural biological processes, it is questionable whether man can permanently modify the ozone layer^{14,15}.

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Evidence for role of m⁷G^{5'}-phosphate group in recognition of eukaryotic mRNA by initiation factor IF-M₃

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7-methylguanosine 5'-monophosphate inhibits protein synthesis in a fractionated, messenger-dependent, reticulocyte cell-free system. This compound also inhibits binding of histone mRNA to reticulocyte ribosomes as well as interaction of VSV mRNA and histone mRNA but not EMC virus RNA with purified initiation factor IF-M₃. These studies provide evidence that the role of 7-methylguanosine in the mechanism for initiation of eukaryotic mRNA translation may be related to specific recognition of mRNA by initiation factor IF-M₃.

WITHIN the past two years a number of investigators have identified a unique 5'-triphosphate linkage of 7-methylguanosine (m⁷G) to the 5'-terminal nucleotide of various eukaryotic mRNAs¹⁻¹². It has also been reported⁷ that chemical removal of m⁷G from rabbit globin mRNA and the absence of either the 7-methyl group or m⁷G in reovirus mRNA¹³ results in reduced ability of these RNAs to serve as templates for protein synthesis. Hickey *et al.*¹⁴ have found in a wheat germ cell-free system that 7-methylguanosine-5'-monophosphate (m⁷G^{5'}p) inhibits translation of several eukaryotic mRNAs containing the 5'-terminal m⁷Gppp... group. Translation of satellite tobacco necrosis virus RNA, a plant virus RNA with a free 5'-terminal phosphate (see ref. 14), was not, however, inhibited by m⁷G^{5'}p. From these and other studies it was concluded that m⁷G^{5'}p acted as an inhibitor of mRNA addition to the initiation complex¹⁴.

In the present study we have tested the effects of m⁷G^{5'}p on protein synthesis in the rabbit reticulocyte cell-free system. In preliminary experiments m⁷G^{5'}p was added to unfractionated reticulocyte lysate. In these conditions, there was only a slight inhibition of protein synthesis at high concentrations of this nucleotide (1.25 mM m⁷G^{5'}p produced only a 15% inhibition). This is in contrast to our previous results with unfractionated wheat germ extract in which 0.125 mM m⁷G^{5'}p inhibited mRNA translation by more than 90% (ref. 14). A major difference between these systems is that wheat germ extract is dependent on exogenous mRNA for protein synthesis, whereas reticulocyte lysate contains large quantities of endogenous mRNA already associated with polysomes. We have, therefore, used a fractionated, reticulocyte mRNA and initiation factor dependent, cell-free system to study the mechanism of inhibition of protein synthesis by m⁷G^{5'}p. With this system under the direction of homologous reticulocyte mRNA, m⁷G^{5'}p produces a marked inhibition of protein synthesis. In addition, m⁷G^{5'}p specifically inhibits mRNA from entering into a stable 40S or

80S "initiation complex" with reticulocyte ribosomal subunits. Finally, we show that m⁷G^{5'}p inhibits the interaction of certain mRNAs with purified reticulocyte initiation factor IF-M₃.

Inhibition of protein synthesis

A cell-free protein synthesising system, dependent on exogenous mRNA and initiation factors, was prepared from rabbit reticulocytes by treating polysomes with low concentrations of pancreatic RNase, followed by washing the ribosomes with 0.5 M KCl and sedimenting them through a 1.0 M sucrose cushion¹⁵. Other reticulocyte components were prepared as reported previously¹⁶. This system has been characterised previously¹⁵ and demonstrates all the steps necessary for synthesis of authentic polypeptides with relatively high efficiency and linear incorporation of amino acids into protein for up to 1 h (ref. 16). With saturating levels of exogenous reticulocyte poly(A)⁺ mRNA and initiation factors, m⁷G^{5'}p produced a marked reduction in ¹⁴C-leucine incorporation (Fig. 1). Inhibition of protein synthesis by m⁷G^{5'}p was not reversed by threefold excess initiation factors added separately or as a crude mixed factor preparation. Little inhibition was observed with either 7-methylguanosine or 7-methylguanosine-2(3')-monophosphate (Fig. 1). Since globin mRNA has been found to contain 5'-terminal m⁷Gppp... (refs 7 and 13), these results suggest that inhibition of exogenous mRNA translation by m⁷G^{5'}p is related to a structural homology between m⁷G^{5'}p and the 5'-terminus of this eukaryotic mRNA.

Inhibition of initiation complex formation

To study the mechanism of inhibition of protein synthesis by m⁷G^{5'}p, purified initiation factors and radioactive mRNAs have been used. In this way the interaction of mRNA with ribosomal subunits and initiation factors could be followed directly. For this purpose we have used a radioactive cellular mRNA, histone mRNA, which was shown previously to be translated faithfully by the reticulocyte cell-free system¹⁸⁻¹⁹. ³H-histone mRNA was incubated with unfractionated rabbit reticulocyte lysate in protein synthesis conditions but in the presence of 0.2 mM sparsomycin to inhibit movement of labelled mRNA into large polyribosome structures (L.A.W., M. C. Williams, and C. B., unpublished). After a brief incubation at 30 °C, ribosomes and ribosomal subunits were separated by sucrose gradient centrifugation. Association of mRNA with ribosomes was determined by the appearance of label in sucrose gradient fractions compared with the ribosome A₂₆₀ profile. Binding of ³H-histone mRNA to reticulocyte ribosomes occurred rapidly in these conditions (Fig. 2a) and 68% of the added label sedimented in the 80S region after 2 min of incubation. Little ³H-material appeared in the 40S subunit region,

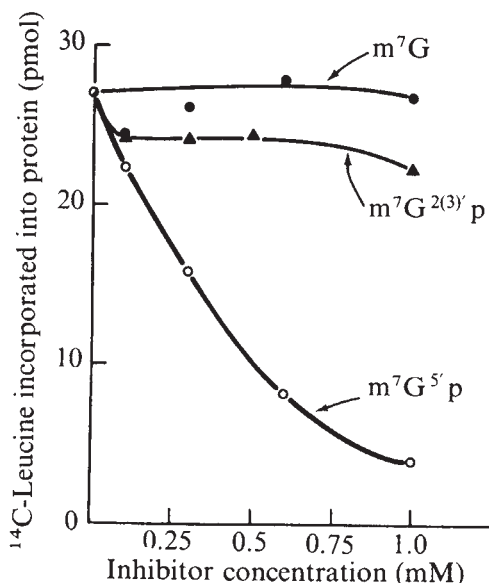


Fig. 1 Inhibition of protein synthesis in reticulocyte messenger-dependent cell-free system by 7-methylguanosine 5'-monophosphate. The preparation of rabbit reticulocyte lysate, 0.5 M KCl-washed ribosomes, 105,000g supernatant protein and crude initiation factors was as previously reported¹⁶. Ribosomes were rendered dependent on exogenous mRNA by incubation with very low levels of pancreatic RNase as previously described¹⁵. Messenger RNA (RNA which contains a 3'-terminal poly(A) sequence) was prepared from reticulocyte polysomes by standard procedures, including multiple extractions with phenol-chloroform-isoamyl alcohol and adsorption of poly(A)⁺ material to oligo(dT)-cellulose according to the method of Aviv and Leder¹⁷. For the complete system, incubations in a total volume of 50 μ l were performed at 30 °C for 30 min and contained 15 mM Tris-HCl, pH 7.4, 80 mM KCl, 3 mM MgCl₂, 1 mM ATP, 0.2 mM GTP, 3 mM phosphoenolpyruvate, 0.2 IU of pyruvate kinase (rabbit muscle), 1 mM dithiothreitol, 80 μ M L-amino acids minus leucine, L-¹⁴C-leucine (specific activity 80 mCi mmol⁻¹), 0.1 A₂₆₀ unit of 0.5 M KCl-RNase treated reticulocyte ribosomes, 0.06 A₂₆₀ units deacylated rabbit liver tRNA, 300 μ g of 105,000g supernatant protein, 150 μ g of crude reticulocyte initiation factors, and 0.2 A₂₆₀ units of partially purified reticulocyte mRNA. Where indicated, 7-methylguanosine (m⁷G) (●), 7-methylguanosine 2(3')-monophosphate (m⁷G^{2(3')p}) (○), or 7-methylguanosine 5'-monophosphate (m⁷G^{5'p}) (▲) were added to the complete system. Reactions were stopped by the addition of ice-cold 10% trichloroacetic acid and protein synthesis determined by incorporation of ¹⁴C-leucine into hot trichloroacetic acid-insoluble material¹⁸.

and unbound ³H-histone mRNA remained near the top of the gradient. In a control experiment ³H-histone mRNA was added to an incubation mixture kept at 0 °C. This did not result in binding of mRNA to ribosomes or ribosomal subunits (data not shown). Addition of 0.25 mM m⁷G^{5'p} to the incubation mixture (Fig. 2b) resulted in a 36% reduction of ³H-histone mRNA binding to ribosomes and 1 mM m⁷G^{5'p} produced a 75%

reduction in ³H-histone mRNA binding (Fig. 2c). m⁷G^{5'p} did not cause a shift in ³H-histone mRNA binding from the 80S to the 40S region (Fig. 2b, c).

Various investigators have reported that Met-tRNA_f forms a ternary complex with GTP and a specific initiation factor IF-MP²⁰ (also referred to as IF-I^{21,22}, IF-E2²³, and IF-L3²⁴). Formation of this complex is thought to lead to Met-tRNA_f association with the 40S subunit independent of mRNA, and it has been reported that Met-tRNA_f-40S subunit interaction is required for incorporation of mRNA into the "initiation complex"²³⁻²⁵. To measure formation of the Met-tRNA_f-40S complex, purified rabbit reticulocyte Met-tRNA_f charged with ³H-methionine (3 Ci mmol⁻¹) and the sucrose gradient assay described by Darnborough *et al.*²⁵ were used. As shown in Fig. 3, binding of ³H-Met-tRNA_f to the 40S ribosomal subunit is not inhibited by m⁷G^{5'p}. From these studies (Fig. 2 and Fig. 3) we conclude that m⁷G^{5'p} inhibits protein synthesis by preventing mRNA from forming a stable complex with ribosomes or ribosomal subunits, but this does not occur by inhibition of Met-tRNA_f-40S subunit complex formation.

Effect of m⁷G^{5'p} on IF-M3-mRNA interaction

To determine whether m⁷G^{5'p} might inhibit protein synthesis by competing with mRNA for a common binding site on one of the initiation factors, a direct assay of mRNA-protein interaction was used. Hellerman and Shafritz²⁶ reported that reticulocyte initiation factor IF-MP forms a complex with ³H-labelled rabbit globin mRNA. This complex was demonstrated by its retention on a nitrocellulose filter, in conditions in which free mRNA passed through the filter. A similar complex between IF-MP and bacteriophage R17-RNA has been reported by Kaempfer²⁷. More recently, using labelled vesicular stomatitis virus (VSV)-mRNA or duck globin mRNA of much higher specific activity, we have found that reticulocyte initiation factors IF-M₃ and IF-M_{2A} also form complexes with mRNA. Other purified reticulocyte initiation factors form such complexes to a much lesser extent (to be reported in detail elsewhere).

For experiments of this type, as well as these reported below, highly purified initiation factors are required. Details for the purification of these factors have been published^{20,28-30} and materials used in the present study were prepared as follows: IF-MP was purified up to and including step 5 in ref. 20, IF-M_{2A} up to and including step 6 in ref. 28, IF-M_{3B} up to and including step 5 in ref. 29, and IF-M₃ up to and including step 5 in ref. 30, plus an additional purification by ion exchange chromatography with phosphocellulose in HEPES buffer at pH 6.8. This IF-M₃ preparation contains predominantly one polypeptide component (~80-90%) of molecular weight ~80,000 on sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis and seems to correspond to factor IF-E6 of Staehelin *et al.*²³.

In the present study, we have examined mRNA-protein complex formation with ³H-histone mRNA, ³H-VSV mRNA

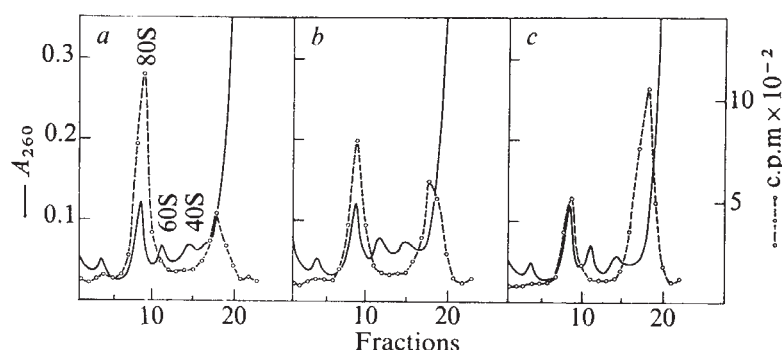


Fig. 2 Inhibition of histone mRNA binding to reticulocyte initiation complexes by 7-methylguanosine-5'-monophosphate. For each 25 μ l incubation of unfractionated reticulocyte lysate, 5,000 c.p.m. of ³H-histone mRNA (specific activity > 200,000 c.p.m. μ g) were added together with 0.1 mM sparsomycin and m⁷G^{5'p}, where indicated. Sparsomycin was added specifically to bind mRNA to 80S initiation complexes, since this drug prevents elongation, but does not inhibit initiation²⁸. At the end of the incubation 0.4 ml of ice-cold 10 mM NaCl, 10 mM Tris pH 7.4 and 1.5 mM MgCl₂ were added and the samples fractionated by 16 h centrifugation at 22,000 r.p.m. on 15-30% sucrose gradients prepared in the same buffer. Fractions of 0.8 ml were collected and counted directly by addition of 15 ml of scintillation fluid containing 1 volume Triton X-100, 2 volumes of 0.8% butyl-PED (CIBA) and 0.3 volume water. a, Control, no m⁷G^{5'p} added. b, 0.25 mM m⁷G^{5'p} added. c, 1.0 mM m⁷G^{5'p} added.

and ^3H -encephalomyocarditis (EMC) virus RNA. Histone mRNA and VSV mRNA contain "blocked", methylated 5'-termini (ref. 31 and B. Moss, personal communication), whereas EMC virus RNA contains an unblocked 5'-terminal structure³². In addition, histone mRNA does not contain 3'-terminal poly(A)³³. As shown in Table 1, complexes can be demonstrated between all three ^3H -mRNAs and initiation factors IF-MP, IF-M₃ and IF-M_{2A} but not with IF-M_{2B}. Inhibition of mRNA-protein complex formation by $\text{m}^7\text{G}^{\text{ppp}}$ is found exclusively with IF-M₃. Assuming a molecular weight of 2.7×10^6 daltons for EMC virus RNA and an average molecular weight of 0.5×10^6 for 12-18S VSV mRNA and roughly equivalent percentages of uridine in these molecules, complex formation between IF-M₃ and EMC virus RNA on a molar basis is approximately one-fourth that obtained with VSV mRNA. In addition, complex formation between ^3H -EMC virus RNA and IF-M₃ is not inhibited by $\text{m}^7\text{G}^{\text{ppp}}$ (Table 1). Since all three mRNAs form complexes with initiation factors IF-MP, IF-M₃ and IF-M_{2A}, a 3'-poly(A) sequence does not seem to be required for the observed phenomena.

It is important to note that highly purified IF-M₃ is required to obtain the observed degree of inhibition with $\text{m}^7\text{G}^{\text{ppp}}$; only 30-40% inhibition is found with less purified material. Inhibition of IF-M₃-mRNA complex formation also seems to be specific for $\text{m}^7\text{G}^{\text{ppp}}$, as other guanosine nucleotides tested do not produce this effect (Table 2). Together with the experiments reported in Figs 2 and 3, these results indicate that inhibition of protein synthesis by $\text{m}^7\text{G}^{\text{ppp}}$ correlates with the ability of this compound to prevent interaction of mRNA with IF-M₃. Presumably, IF-M₃-mRNA interaction is required for mRNA association with the 40S ribosomal subunit, but as yet we have not developed an assay system to test this directly.

Relationship of m^7Gppp . . . to the eukaryotic initiation process

In spite of many recent advances in our understanding of the mechanism of initiation of eukaryotic protein synthesis, little

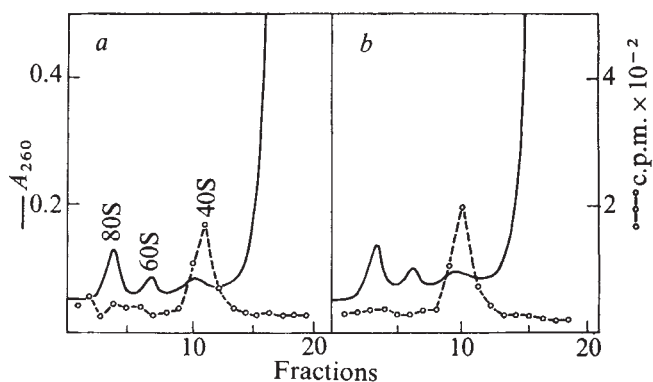


Fig. 3 Binding of ^3H -Met-tRNA_i to the small ribosomal subunit in unfractionated rabbit reticulocyte extract in the absence (a) or presence (b) of $\text{m}^7\text{G}^{\text{ppp}}$. The binding reactions were carried out according to Darnbrough *et al.*²⁶ in 0.1 ml of the complete reticulocyte lysate system. To each incubation was added 0.1 mM sparsomycin and 1 mM $\text{m}^7\text{G}^{\text{ppp}}$ was added to b. After 2 min at 30 °C, 3 pmol of ^3H -Met-tRNA_i (1,500 c.p.m. pmole⁻¹) from rabbit reticulocytes were added for 2 min. The incubations were terminated by the addition of 0.4 ml of ice-cold 10 mM NaCl, 10 mM Tris pH 7.4 and 1.5 mM MgCl₂. The samples were fractionated by centrifugation on 15-30% sucrose gradients in the same buffer for 16 h at 22,000 r.p.m. in the Beckman SW27 rotor. 0.8-ml fractions were collected and counted after addition of 0.25 mg carrier yeast tRNA and precipitation with 1.5 ml of 2% acetyltrimethylammonium bromide. The precipitates were collected on Millipore filters and counted as in Fig. 2.

progress has been made in elucidating how mRNA is recognised by the ribosome or how specific factors might participate in selecting specific mRNAs or particular nucleotide sequences of mRNAs. To approach this problem, we have begun to study the properties of proteins associated with mRNA and the relationship of these proteins to reticulocyte initiation factors. We reported that IF-MP activity is present on messenger

Table 1 Inhibition by $\text{m}^7\text{G}^{\text{ppp}}$ of binding of ^3H -uridine-labelled mRNAs to reticulocyte initiation factors

Factor	^3H -VSV mRNA bound (c.p.m.)		^3H -histone mRNA bound (c.p.m.)		^3H -EMC virus RNA bound (c.p.m.)	
	Control	+ $\text{m}^7\text{G}^{\text{ppp}}$	Control	+ $\text{m}^7\text{G}^{\text{ppp}}$	Control	+ $\text{m}^7\text{G}^{\text{ppp}}$
IF-MP	2,710	2,580	872	919	16,588	13,917
IF-M ₃	1,930	643	796	69	18,261	22,059
IF-M _{2A}	2,618	2,411	452	360	40,429	36,614
IF-M _{2B}	126	—	23	—	197	284

Binding of ^3H -VSV mRNA, ^3H -histone mRNA and ^3H -EMC virus RNA to purified reticulocyte initiation factors was determined by the nitrocellulose filter assay reported previously²⁶. ^3H -VSV mRNA was prepared from virus-infected HeLa cells incubated with ^3H -uridine by the method of Grubman *et al.*³⁵. This RNA contains poly(A), sediments between 12S and 18S, and has been shown to have $\text{m}^7\text{G}^{\text{ppp}}$ at the 5'-terminus³¹. ^3H -histone mRNA was prepared from HeLa cells synchronised in S phase and incubated with ^3H -uridine. The histone mRNA was isolated by phenol extraction and repeated sucrose gradient centrifugation (L. A. W., M. C. Williams, and C.B., unpublished). Histone mRNA, labelled with ^3H -methylmethionine and isolated as described above, has been shown to have a blocked 5'-terminus (B. Moss, personal communication). The ^3H -methylhistone mRNA was enzymatically digested and analysed by chromatography on DEAE-cellulose in 7 M urea at pH 7.6. About 80% of the c.p.m. applied were eluted with a charge of -5 or -6, which is the position expected for blocked 5'-termini³⁶. Encephalomyocarditis (EMC) virus RNA was prepared from HeLa cells. The cells were concentrated to 4×10^7 ml⁻¹ in serum-free MEM medium containing 14 mM HEPES buffer pH 7.4 and 2 mM glutamine. EMC virus was added at a multiplicity of 10 PFUs cell⁻¹ for 15 min at 23 °C. The culture was diluted tenfold with warm medium containing 7% foetal calf serum and 5.5 μg ml⁻¹ actinomycin D. After 30 min at 37 °C ^3H -uridine (26.2 Ci mmol⁻¹) was added to a final concentration of 50 μCi ml⁻¹. After 14 h, the culture was frozen and thawed three times and centrifuged 10 min at 10,000g. The supernatant was neutralised with 1 M Tris and made 6% in polyethylene glycol. The precipitate which formed after stirring for 16 h at 4 °C was collected by 15 min centrifugation at 15,000g and resuspended in 2 ml of 0.1 M NaCl, 10 mM Tris pH 7.5 and 10 mM mercaptoethanol. This suspension was incubated for 20 min at 37 °C with 50 μg ml⁻¹ of trypsin and SDS added afterwards to 0.5%. Virus was purified by 2-h centrifugation at 150,000g through a 2-ml layer of 15% sucrose in SDS-buffer³⁷. The pellet was resuspended in SDS-buffer and the RNA extracted with phenol-chloroform³⁷. RNA was recovered by ethanol precipitation and was purified by 15-h centrifugation at 25,000 r.p.m. on a 15-30% sucrose gradient. The 35S viral RNA was precipitated with ethanol and reprecipitated twice. The EMC virus RNA has a specific activity of 380,000 c.p.m. μg^{-1} . Incubations in a total volume of 50 μl were performed at 23 °C for 5 min and contained 20 mM Tris-HCl, pH 7.4, 100 mM KCl, 1 mM dithiothreitol and 0.53 μg IF-MP, 3.5 μg IF-M₃, 1.1 μg IF-M_{2A} or 0.99 μg IF-M_{2B}. In each case, 4,000-5,000 c.p.m. ^3H -VSV mRNA (specific activity 450,000 c.p.m. per A_{260} unit), 3,000 c.p.m. of ^3H -histone mRNA (specific activity > 200,000 c.p.m. μg^{-1}) or 60,000 c.p.m. ^3H -RMC virus RNA (specific activity 380,000 c.p.m. μg^{-1}) was included as substrate and reactions were performed either in the absence (control) or presence of 1.0 mM $\text{m}^7\text{G}^{\text{ppp}}$. Reactions were stopped by the addition of 3 ml of ice-cold buffer solution (20 mM Tris-HCl, pH 7.4, 100 mM KCl). RNA-protein complexes were collected on HAWP 0.45 μm pore size, 25 mm diameter, nitrocellulose filters (Millipore) and rinsed three times with 3 ml of the same buffer. The filters were dissolved in 10 ml Bray's solution³⁸ and counted by liquid scintillation spectroscopy at 23% efficiency for ^3H . Blanks of 175 c.p.m., 84 c.p.m. or 671 c.p.m., representing isotope retention on the nitrocellulose filter in the absence of added protein factors, were subtracted from the appropriate values.

* For experiments with ^3H -EMC virus RNA, the amount of the protein factors was reduced to one-third that used for the other mRNAs.

ribonucleoprotein particles (mRPNs) obtained from reticulocyte polysomes and that purified IF-MP can interact with globin mRNA to form an mRNA-protein complex²⁶. In the present studies, we have found that in addition to IF-MP, purified initiation factors IF-M₃ and IF-M_{2A} can also form complexes with mRNA. Of the various factors tested, however, only the interaction of IF-M₃ with mRNA is inhibited by m⁷G⁵p. This inhibition requires both the 7-methyl and the 5'-phosphates moieties, and the requirements for inhibition of IF-M₃-mRNA complex formation are the same as those for inhibition of overall protein synthesis.

The amount of m⁷G⁵p required for inhibition of either protein synthesis or IF-M₃-mRNA interaction is far in excess of the amount of mRNA present in the system (greater than 1,000/1). Therefore, the interaction of m⁷G⁵p with IF-M₃ seems to represent a low affinity binding and the 5'-terminal region of natural mRNAs recognised by IF-M₃ may contain additional nucleotides. Since we are limited in the amount of specific initiation factors we can add to the cell-free system, the high ratio of m⁷G⁵p to IF-M₃ may explain our inability to obtain reversal by excess IF-M₃. It should also be noted that endogenous protein synthesis with unfractionated reticulocyte lysate is only slightly inhibited by m⁷G⁵p. In the reticulocyte lysate system, however, m⁷G⁵p does inhibit association of exogenous histone mRNAs with ribosomes (Fig. 2), as well as translation of exogenous histone mRNA (our unpublished observations). At the moment, we have no explanation for the low inhibition of endogenous protein synthesis by m⁷G⁵p in reticulocyte lysate. One possible interpretation of these findings is that endogenous mRNA in reticulocyte lysate is already associated with specific proteins (such as IF-M₃) and that translation of performed mRNA protein complexes cannot be inhibited by m⁷G⁵p, whereas translation of added deproteinised mRNA can be. We hope to test this hypothesis in future experiments by comparing the effect of m⁷G⁵p on translation of exogenous reticulocyte mRNA compared with reticulocyte mRNP in the fractionated messenger dependent cell-free system.

serve as a specific recognition signal for initiation of eukaryotic protein synthesis.

As indicated above, the affinity of natural mRNA for IF-M₃ is probably much greater than that observed with m⁷G⁵p. Therefore, the present studies should not be taken to imply that m⁷G⁵p represents the complete sequence of mRNA recognised by IF-M₃ or that IF-M₃ binds solely to this region of mRNA. The reduced but yet significant binding of EMC virus RNA to IF-M₃ and the lack of inhibition of this complex by m⁷G⁵p would support such a conclusion. These results are also consistent with the observation of Staehelin *et al.*²³, that threefold greater quantities of factor IF-E6 (IF-M₃) are required for translation of EMC virus RNA compared with rabbit globin mRNA. In future studies, we hope to better define the process by which IF-M₃ interacts with mRNA by comparing the affinity of various ³H-labelled nucleotides for this protein (m⁷G⁵p, m⁷G⁵pp, m⁷G⁵ppp, m⁷G⁵pppNp, for example), and to determine the relationship of this event to other mRNA factor interactions which may be involved in the eukaryotic initiation mechanism. Since it has been shown recently that *in vitro* translation of "unblocked" mRNAs, such as satellite tobacco necrosis virus (STNV) RNA¹⁴ and encephalomyocarditis (EMC) RNA L. A. W., E. R. Feman, E. D. Hickey, M. C. Williams, and C. B., unpublished, is not inhibited by m⁷G⁵p, it will also be important to determine whether translation of mRNAs not containing 5'-terminal 7-methylguanosine, proceeds through the same or a different mechanism.

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Table 2 Specificity for inhibition of ³H-VSV mRNA-IF-M₃ complex formation by guanosine nucleotides

Inhibitor added	c.p.m. ³ H-VSV mRNA bound	% Inhibition
None	2,260	—
m ⁷ G	1,900	16
m ⁷ G ²⁽³⁾ p	1,965	13
m ⁷ G ⁵ p	800	65
G ⁵ p	2,021	10

For incubation conditions and assay procedure see Table 1. Where indicated, incubations were performed in the presence of 1.0 mM m⁷G, m⁷G²⁽³⁾p, or m⁷G⁵p, G⁵p added before ³H-VSV mRNA.

From the present studies, it seems that association of mRNAs containing m⁷Gppp... with initiation factor IF-M₃ may be essential for binding of certain mRNAs to the ribosome. In addition, inhibition of IF-M₃-mRNA interaction by m⁷G⁵p may be the result of competition between this nucleotide and mRNA for a specific recognition site on IF-M₃. Since m⁷G⁵ppp... has been identified as the 5'-terminal group in various eukaryotic mRNAs, a normal function of IF-M₃ might be to form a complex with certain mRNAs through their 5'-terminal m⁷Gppp... sequence. This would agree with previous findings of Both *et al.*^{13,34} that absence of 7-methylguanosine from the 5'-terminus of certain eukaryotic mRNAs markedly reduces messenger-ribosome complex formation and translation of these messengers and would support the hypothesis of these investigators³⁴ that the m⁷Gppp... region of mRNA may

letters to nature

X-ray outburst from Circinus X-1

DURING observations of the Circinus–Norma region by the rotation modulation collimator (RMC) experiment on board Ariel V, a sudden strong X-ray outburst was detected from Cir X-1. The source appeared on 1976 February 15 at 0500(UT) at an intensity of 20 counts s^{-1} . It gradually brightened to over 40 counts s^{-1} on February 17 at 2200(UT) and then rapidly declined on February 18. Our best position, RA = 229. 19°, Dec = −57.00°, with Error Circle, 0.02° radius (90% confidence), is in excellent agreement with the known position¹ of Cir X-1.

The light curve is shown in Fig. 1. The data have been corrected for instrumental response and source visibility. Data gaps occur whenever the experiment is switched to its 'time' mode of operation, when positional resolution is sacrificed for better time resolution. The data points have a nominal time resolution of ~100 min (1 orbit) except where indicated. The onset of the outburst is extremely rapid. The initial detection in a single orbit gives an intensity of ~20 counts s^{-1} ; analysis of the data from the previous orbit gives an upper limit (3σ) of 5 counts s^{-1} . For compatibility with data preceding and succeeding the outburst and the known times when the source was in the field of view, the transition to the high stage must have occurred between 0400 and 0512 (UT) on 1976 February 15. When the data for the preceding 10 h is summed, we obtain a marginal detection at 2.5 ± 0.8 (1σ) counts s^{-1} . Earlier observations give a 3σ upper limit of 1.2 counts s^{-1} for 1976 February 6.5 to February 14.0.

The source showed a rapid decline in intensity following peak brightness, falling to just above the detector threshold in a few hours. After the data gap, however, the source had recovered to half its maximum intensity, remaining at this level for five hours. It subsequently faded to below the detector threshold. Because about six bright sources are in the field of view of the experiment, it is not possible to recover accurately the absolute intensity of Cir X-1 from the time-mode data. The general

recovery of the source is, however, qualitatively evident in that data. In the half day period after the source went below the threshold the summed data give an upper limit of 2.5 counts s^{-1} for the time averaged intensity; in any single orbit during this period the upper limit is 7 counts s^{-1} .

The photon flux is measured by the RMC experiment in three pulse height channels corresponding to energy ranges 2.9–4.3,

Fig. 1 The light curve of Cir X-1 in 2.9–7.6 keV X rays measured by the RMC experiment on Ariel V. The data are corrected for detector response and source visibility. Error bars are $\pm 1\sigma$ and upper limits are 3σ . Time resolution, if not indicated, is ~100 min (1 orbit). For comparison, 1 count s^{-1} is approximately equivalent to 12 Uhuru counts s^{-1} . TM, time mode.

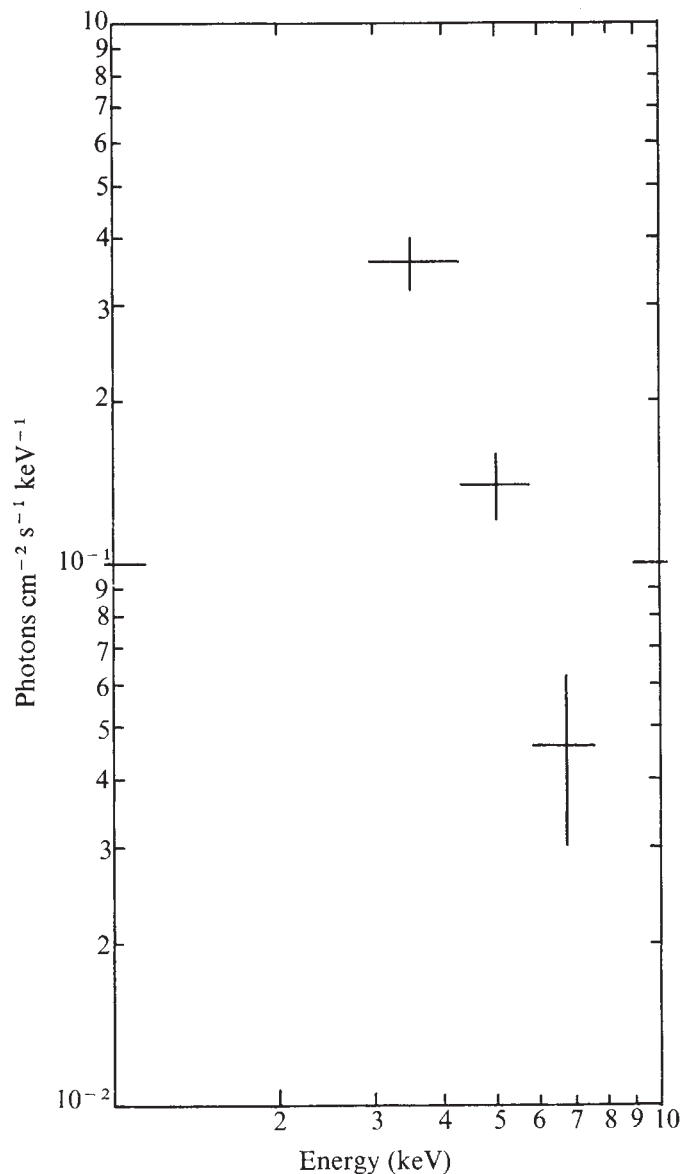
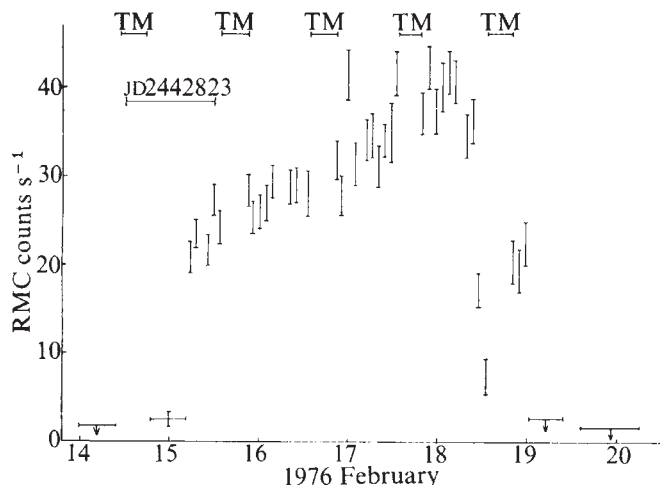


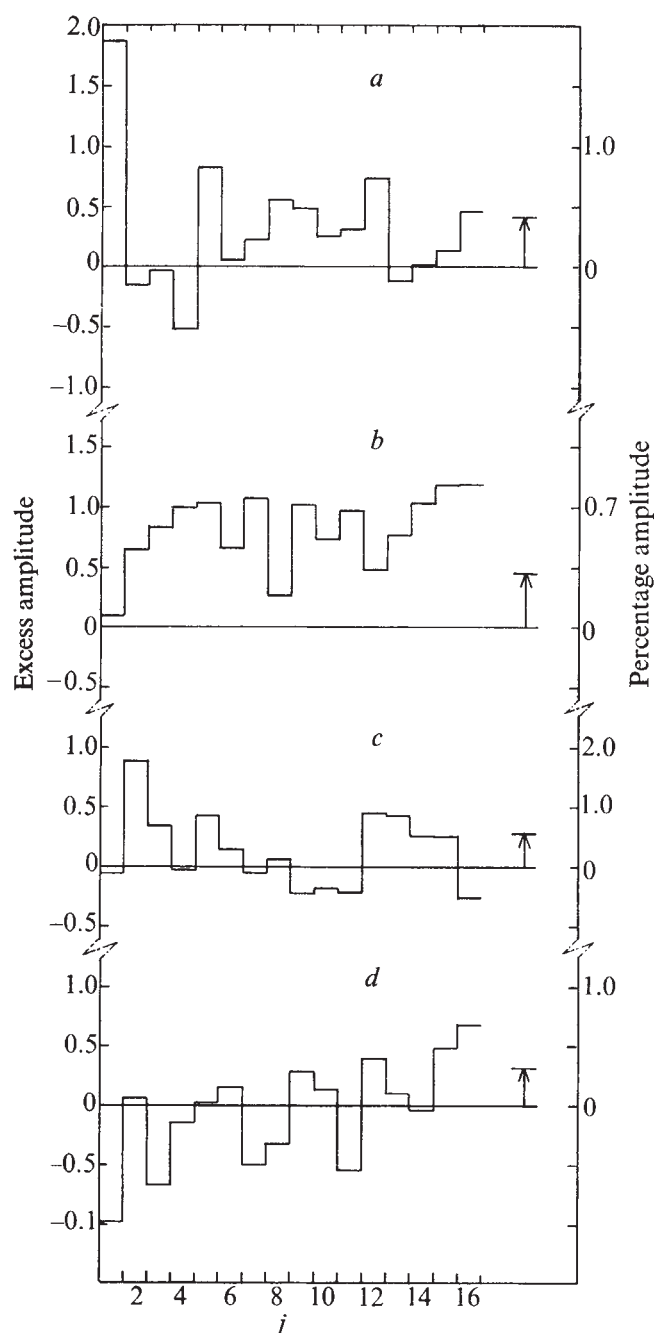
Fig. 2 The X-ray spectrum of Cir X-1 at maximum intensity. Data used are taken from 1976 February 17, 1900(UT) to 1976 February 18 0500(UT). Error bars are $\pm 1\sigma$.

4.3–5.9 and 5.9–7.6 keV respectively. This enables us to compute the photon number spectrum for the source, which is plotted in Fig. 2 for maximum X-ray intensity. Throughout the outburst reported here the spectral data can be fitted by a thermal exponential spectrum with $kT = 1.7$ –2.4 keV at a column density of 10^{22} atoms cm^{-2} . But, the data are too insensitive for good evaluation of the absorption term. Unfortunately

photon statistics are too poor to allow us to follow spectral changes during the decay phase of the outburst, or to measure a spectrum in the period immediately before the high intensity onset.

In the time mode operations indicated in Fig. 1, counts are simply accumulated in consecutive time bins of 32 s. Sequences of 32 bins were selected which were free of passages through regions of high particle background, and free of occultation of the Sun or the source by the Earth. Any trends in the data were determined by a least squares analysis fitting a polynomial, degree 3, and subtracted out, except for the pre-outburst

Fig. 3 Mean power spectra measured by the RMC experiment on Ariel V. The abscissa is the excess amplitude measured in units of the expected amplitude; the corresponding percentage amplitude is indicated. The ordinate, j , is frequency bin number, bin 1 corresponds to 0.00098 Hz, bin 16 corresponds to 0.0156 Hz. *a*, Six sequences of 32 time bins measured on 1976 February 13–14; *b*, nine sequences of 32 time bins measured on 1976 February 15–17; *c*, three sequences of 32 time bins measured on 1976 February 18; *d*, ten sequences of 32 time bins measured on 1976 February 20–21. Arrow on abscissa gives the s.d. $\langle\sigma_p\rangle$.



data. Power spectra were then obtained for each sequence and summed. The results are summarised in Fig. 3. The abscissa is: $(P_j - \langle P \rangle) / \langle P \rangle$ where P_j is the amplitude in the j th frequency bin and $\langle P \rangle$ is that expected from counting statistics from a constant source; $\langle\sigma_p\rangle$ is the expected s.d. from counting statistics.

The pre-outburst mean power spectrum shows a non-random power distribution which we attribute to other sources in our field of view. During the outburst from Cir X-1, excess power density is measured at all frequencies with no single frequency-bin outstanding. The power density in the decay phase of the outburst is much less and approaches that measured in data since the outburst.

The first definite observation² of Cir X-1 was made in 1969. Previous observations give a conflicting view of the sky in that region and source confusion must be assumed. Since then many observations^{1,3–7} of the source show it predominantly at a low intensity, with occasional outbursts to a higher level, with rapid, erratic changes in its behaviour, variable on time scales down to $\ll 1$ s. The model of Cir X-1 as a young runaway binary in a highly elliptical, long period orbit⁸, would predict high X-ray emission from Cir X-1 near periastron. The data of Baity *et al.*⁴, and Davison and Tuohy⁷ suggest an interval of ~ 220 d between times of high X-ray emission. Since the Copernicus observation⁷, SAS-3 has observed Cir X-1 (ref. 8) and our report further extends the base line. Our data give a well determined time at which an outburst occurred, and record a complete cycle of increased emission. The behaviour is consistent with an outburst of high intensity every 220–250 d, but consideration of the reported times of previous outbursts suggests that the phenomenon is not strictly periodic but irregular. Our data are not good enough to confirm any softening of the spectrum with enhanced emission^{1,4}, or to give any new information on changes in absorption during the event². The softness of the spectrum does not conflict with the suggestion that the long term intensity variations of Cir X-1 are due to low energy X rays⁴.

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The infrared spectrum of Nova Cygni 1975

GRASDALEN and Joyce¹ have observed emission lines from Nova Cygni 1975 in the 2 and $3\ \mu\text{m}$ spectral regions. In mid-September 1975, the dominant spectral features were recombination lines of hydrogen. Grasdalen and Joyce noted the presence of a number of additional lines in a later spectrum and identified these with forbidden transitions in highly ionised forms of Mg, Ca, Al, and Si. The prerequisite for formation of such coronal lines is an

Table 1 Infrared line identifications

λ_{obs} (μm)	Peak flux (Jy) September 12, 1975	Peak flux (Jy) October 27, 1975	λ_{pred} (μm)	
1.90	>30	>1.8	1.909	He I 4 ¹ D-3 ¹ P ⁰
1.943	33	1.8	1.945	H Br δ
2.06	30	1.7	2.060	He I 2 ¹ P ⁰ -2 ¹ S
2.11	17	1.0	2.114	He I 4 ¹ S-2 ¹ P ⁰
2.167	52	2.9	2.165	H Br γ
2.86	28	1.6	2.87	H Pf ζ
3.06	30	1.7	3.04	H P η
3.27	32	1.8	3.30	H Pf δ , He I 5 ¹ P ⁰ -4 ¹ S
3.50	27	1.5	unidentified	
3.73	37	2.1	3.74	H Pf γ
4.02	100	5.6	4.05	H Br α , He I 5 ¹ P ⁰ -4 ¹ D
1.959		2.0	1.954	He I 4 ³ P ⁰ -3 ³ D
2.040		1.9	2.042	He I 6 ³ P ⁰ -4 ³ S
2.32		0.3	2.321	He I 13 ³ P ⁰ -5 ³ S
2.474		3.8	2.473	He I 6 ³ D-4 ³ P ⁰
2.879		2.2	2.854	He I 5 ³ P ⁰ -4 ³ S
3.021		8.2	3.033	He I 10 ³ F ⁰ -5 ³ D
3.18		0.8		
3.661		3.9	3.678	He I 13 ³ P ⁰ -6 ³ S
3.72		3.9	3.703	He I 5 ³ D-4 ³ P ⁰
3.92		2.0	3.910	He I 14 ³ D-6 ³ P ⁰

emitting region of low density and extremely high temperature, $T \sim 10^6$ K, which, moreover, must span a considerable range of temperature in order for species such as Al V, VI, VIII and IX to exist simultaneously. This would seem to preclude an isothermal shock as the mechanism responsible for a high temperature region in Nova Cygni. We propose a more conservative identification of these new lines, namely that they are all lines of He I with one possible exception. Furthermore, their sudden appearance and increase in relative intensity are in accord with somewhat more temperate conditions ranging from $T \sim 10^4$ K in September 1975 to $T \sim 10^5$ K in October. This identification is also more consistent with the known behaviour of very fast novae, which rarely exhibit optical coronal emission lines².

Table 1 lists the wavelengths of the features observed by Grasdalen and Joyce and our suggested identifications. The observed wavelengths are from the table of Grasdalen and Joyce or are estimated from their figures. The peak fluxes have been estimated from the published spectra and from the scaling procedure used in the original analysis. These fluxes are presented to illustrate the change in relative fluxes between September 12 and October 27; they should not be taken literally. The predicted wavelengths have been taken from standard references^{3,4}.

The strongest lines in the September spectrum were correctly identified by Grasdalen and Joyce as Brackett and Pfund series lines of hydrogen. As many as five lines of He I also appear, although two are blended with stronger H lines. We suggest that these are not recombination lines, but rather the result of pumping of metastable He I (2¹S) by the very broad, very strong hydrogen Balmer continuum short of 3,646 Å. This pumping occurs through the transition 2¹S-5¹P⁰ at 3,614 Å, and the 5¹P⁰ state decays through a cascade which includes all the singlet transitions listed in Table 1. Table 2 lists additional lines in the visible and

near infrared which would also appear as part of this fluorescence process, and which should share the intensity variation exhibited by the infrared lines. The absence of detectable triplet transitions in He I (which should dominate the recombination spectrum if He were ionised to the same degree as H) is consistent with a collision-excited plasma with $T \sim 10^4$ K in September.

According to our interpretation, the October spectrum shows strong He I recombination lines. The agreement between observed and predicted wavelengths is at least as good as that found by Grasdalen and Joyce for the coronal ions, and all the more striking since only one species is involved. The relative intensities of the suggested He I lines are in harmony with transition probabilities⁴ and qualitative expectations from recombination theory. Note that the He I lines are as strong as the H lines in the October spectrum. This can occur in a plasma at $T \sim 10^5$ K in which H and He are present in normal cosmic abundance (10-15% He by number) and are both singly ionised. At such temperatures, He recombines nearly ten times faster than H because of dielectronic recombination and should thus exhibit an appropriately stronger recombination cascade. A temperature of $T \sim 10^5$ K is further supported by the appearance of strong emission from the N III 4,640 Å blend in October because at $T = 10^4$ N III becomes the dominant stage of nitrogen⁵. This interpretation of the infrared spectrum of Nova Cygni is clearly subject to further observational tests through examination of He I lines in visible spectra obtained during the same time period. Not only the physical evolution of the emitting region, but also the helium abundance may be determined from such studies. A more detailed analysis of the recombination spectra in Nova Cygni 1975 will be published elsewhere.

The existence of regions in Nova Cygni with $T \sim 10^5$ K seems physically reasonable. Although the optical decline was probably paralleled by a true decrease in luminosity, some energy source must have remained to provide for the substantial optical and infrared flux. From the observations reported by Gallagher and Ney⁶, the luminosity can be estimated at $\sim 10^4 L_{\odot}$ in late October. Both theoretical and empirical arguments suggest that, by this stage, the nova remnant would have a small radius and correspondingly high effective temperature (G. T. Bath and G. Shaviv, and J. S. Gallagher and S. G. Starrfield, unpublished). The He I emission might then originate in a dense, hot medium near the underlying star.

Table 2 He I lines excited by Balmer continuum

Transition	Wavelength (Å)
4 ¹ S-2 ¹ P ⁰	5,047
4 ¹ D-2 ¹ P ⁰	4,921
5 ¹ P ⁰ -3 ¹ S	11,013
5 ¹ P ⁰ -3 ¹ D	12,756
3 ¹ P ⁰ -2 ¹ S	5,015
3 ¹ S-2 ¹ P ⁰	7,281
3 ¹ D-2 ¹ P ⁰	6,678

Finally, we call attention to the unidentified line at $3.50\ \mu\text{m}$ and to the line at $3.18\ \mu\text{m}$, which appear not to be He I lines. The latter may indeed be [Ca IV] as suggested by Grasdalen and Joyce since Ca exists predominantly in this stage of ionisation at our inferred temperature.

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Can cosmic clouds cause climatic catastrophes?

SEVERAL authors¹⁻³ have discussed whether past immersion of the Solar System in dense interstellar matter (ISM) might have left observable imprints on the Earth. Accretion cannot affect the solar constant significantly unless the surrounding ISM attains densities of 10^5 – $10^7\ \text{cm}^{-3}$ (refs 2 and 3). We demonstrate here, however, that a much more modest ISM density of 10^2 – $10^3\ \text{cm}^{-3}$ (a value typical of dense interstellar clouds in spiral arms) would prevent the solar wind from reaching the Earth, and propose that such a change in the Earth's space environment may trigger drastic climatic changes.

Suppose that the Sun moves with velocity v_∞ through ISM of density n_∞ and temperature T_∞ . The solar wind can be envisaged as a supersonic radial flux of fully ionised particles with density n_E and velocity v_E at the Earth ($r = 1\ \text{AU}$). If there were no mixing or interpenetration between solar wind and

ISM, there would be a contact discontinuity with the rough shape shown in Fig. 1, located at a distance r_* from the Sun in the 'upstream' direction. (In practice, the SW may be shocked before reaching r_* ; and the ISM, if its properties were sufficiently 'fluid-like', might pass through a shock outside r_* .)

In the idealised case shown in Fig. 1, r_* has the value at which the 'ram pressure' $\propto n_E v_E^2 / r_*^2$ of the solar wind balances the pressure of the infalling ISM. We can estimate this as follows. The speed of a typical ISM particle at distance r is

$$v(r) \simeq \left(v_\infty^2 + v_{\text{th}}^2 + \frac{v_{\text{esc}}^2}{r/\text{AU}} \right)^{1/2}$$

where $v_{\text{esc}} \simeq 42\ \text{km s}^{-1}$ is the escape speed from the Sun at 1 AU. In most cases of interest, v_∞ will be greater than the thermal velocity $v_{\text{th}} \simeq (T_\infty/100)^{1/2}\ \text{km s}^{-1}$; and we therefore neglect v_{th} to keep our discussion short. If the mean free path in the ISM is so large that, until reaching r_* , each particle follows an independent hyperbolic orbit under the influence of the Sun's gravity, then the density at a distance r ahead of the Sun is $n(r) \simeq n_\infty v(r)/v_\infty$ due to 'gravitational focusing'. Defining r_* by the equality $n(r_*) [v(r_*)]^2 = n_E v_E^2 / r_*^2$, we obtain

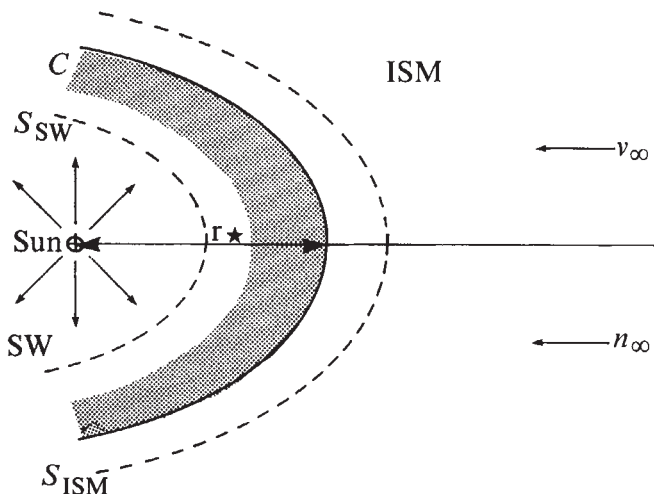
$$r_* \propto \begin{cases} n_\infty^{-1/2} v_\infty^{-1} & \left[r_* \gtrsim \left(\frac{v_{\text{esc}}}{v_\infty} \right)^2 \right] \\ n_\infty^{-2} v_\infty^{-2} & \left[r_* \lesssim \left(\frac{v_{\text{esc}}}{v_\infty} \right)^2 \right] \end{cases} \quad (1a) \quad (1b)$$

Once n_∞ becomes large enough to bring r_* below $(v_{\text{esc}}/v_\infty)^2\ \text{AU}$, the gravitational focusing causes r_* to depend much more sensitively on n_∞ . When the inflow of the ISM is fluid-like and standard accretion theory can be used, the density enhancement goes as $(v/v_\infty)^3$ rather than just as (v/v_∞) , so the dependence is more sensitive still. Taking $v_E = 450\ \text{km s}^{-1}$ and $n_E \simeq 5\ \text{cm}^{-3}$, equation (1b) implies that r_* would lie within 1 AU when $n_\infty \gtrsim 100 (v_{\text{esc}}/10\ \text{km s}^{-1})^2\ \text{cm}^{-3}$. Allowance for fluid-like behaviour of the ISM would lower the required n_∞ further, but we note that it is in any case comparable with 'typical' interstellar cloud densities. (If the ISM outside r_* behaved as a fluid, we would obtain an even more drastic result than equation (1b): r_* would be forced down to zero (the solar wind would be entirely 'snuffed out') whenever r_* were forced within a value $\sim (v_{\text{esc}}/v_\infty)^2\ \text{AU}$, because within that radius the ram pressure provided by radial accretion is $\propto r^{-5/2}$, which is steeper than the r -dependence of the solar wind pressure.

When the ISM is mainly neutral, hydrogen is acted on by radiation pressure from the solar Lyman α (refs 4 and 5). This yields a repulsive force comparable to the Sun's gravitational attraction, thereby reducing the gravitational focusing. Significant photoionisation of neutral interstellar hydrogen (H I) would occur within a few AU; and any penetration of ionised ISM into the solar wind would be prevented by plasma instabilities and the transverse solar wind magnetic field. If some ISM encounters the solar wind while still neutral, the contact discontinuity will be replaced by a region of interpenetration whose thickness is governed by the length scale for ionising the remaining neutral particles. Once ionised, such particles rapidly join the bulk solar wind flow. This flow will be slowed down by charge exchange reactions in the interpenetration region⁴.

The character of the transition region at $r \lesssim r_*$ is rather complicated; and effects such as radiation pressure and charge exchange would modify the simple formulae we have given for r_* . We find, however, that the ISM density required to reduce r_* below 1 AU exceeds that inferred from equation (1) by a factor of no more than 2 or 3 (M. Begelman, in preparation); moreover, the corrections for fluid-like behaviour outside r_* (which

Fig. 1 Schematic depiction of the termination of the solar wind (SW) by the interstellar medium (ISM). With respect to the Sun, the ISM has streaming speed v_∞ , density n_∞ , at $r \rightarrow \infty$. The termination radius r_* is found by balancing ram pressure on either side of an imaginary contact discontinuity C. If the ISM is largely neutral, r_* is the outer boundary of a region of interpenetration (shaded). In the text, we show that the value of n_∞ necessary to make r_* smaller than 1 AU never exceeds $n_\infty \simeq 100 (v_\infty \simeq 10\ \text{km s}^{-1})^2\ \text{cm}^{-3}$ by more than a factor of 2 or 3, regardless of the ionisation level of the ISM. Allowance for possible shock discontinuities S_{SW} and S_{ISM} would reduce the required n_∞ . Clouds populating the spiral arms of the Galaxy may have sufficient densities to prevent the solar wind from reaching the Earth.



become more important for high n_{∞}) tend to reduce the required density. Of course r_* is measured along the line of stagnation. The termination distance in the plane containing the Sun and perpendicular to the line of stagnation is, however, unlikely to be much more than $\sim 2r_*$. Therefore, if $r_* \lesssim 0.5$ AU the Earth will spend part of each year out of reach of the solar wind. If the solar velocity relative to the ISM makes a large angle with the plane of the ecliptic, the Earth may be entirely cut off from the solar wind.

The ISM attains densities $\gtrsim 10^2 \text{ cm}^{-3}$ in H I clouds, in regions of star formation (for example the Orion Nebula)^{6,7}, and in thin sheets swept up by supernova remnants or by the winds of O or B stars^{8,9}. These dense regions would be concentrated in spiral arms. The Solar System traverses an arm every $(1-3) \times 10^8$ yr (this figure being uncertain because we know neither the speed of the spiral wave, nor how regular or long-lived the spiral pattern is); and during each traversal its orbit may encounter several such dense regions. McCrea^{2,3} has suggested that there may be a causal connection between spiral arm traversals and the quasi-periodic epochs of glaciation inferred from the geological record. He tentatively relates the Quaternary glaciation to the Sun's passage into the Orion Arm. But the specific mechanism that he proposed—an alteration of the solar constant caused by accretion—requires an interstellar cloud of density 10^5 – 10^7 cm^{-3} , and such clouds are so compact and unusual that the Sun would have little chance of encountering even one.

There is now an impressive array of evidence suggesting that solar activity influences the Earth's climate through the effects of the solar wind. Immersion in the less extreme and more widespread ISM regions with density 10^2 – 10^3 cm^{-3} can prevent the solar wind from reaching the Earth. This circumstance therefore suggests a possible physical basis for McCrea's conjectured link between spiral arms and terrestrial climate which obviates the need for the extreme ISM densities required by his specific accretion mechanism.

Exclusion of the Earth from the 'heliosphere' might have four major effects: (1) If the ISM were largely neutral at 1 AU, the Earth would be bathed in an enhanced background of cool neutral material: this material could penetrate the magnetosphere and join the upper ionosphere, perhaps significantly changing its composition over periods of $\gtrsim 10^6$ yr. If charge exchange reactions were important in the interpenetration zone, neutral hydrogen would flow past the Earth at solar wind speeds. These particles would also penetrate the magnetosphere, and probably cause ionisation by collisions in the ionosphere. (2) Ions in the vicinity of the Earth would typically have speeds much lower than those of typical solar wind particles. This would affect the rate at which they could be trapped by the magnetosphere, as well as the rate at which trapped particles could be funnelled into the atmosphere over the polar regions. (3) In the absence of solar wind modulation, an enhanced flux of low energy interstellar cosmic rays would reach the Earth. (4) The Earth's magnetosphere would be isolated from the solar magnetosphere. This would seriously inhibit bursts of energetic particles, emitted in such events as solar flares, from reaching the Earth, and is perhaps the most crucial effect.

There is no theory which predicts what effects any of these changes might have on the climate. King^{10,11} and others¹² have found correlations between weather and local short term variations in the geomagnetic field, and direct correlations have been found between atmospheric circulation patterns and crossings of solar wind sector boundaries¹³. These results, suggesting that the lower atmosphere somehow reacts sensitively to the solar wind, are consistent with studies of long term correlations, which take into account migration of the Earth's magnetic poles and polarity reversals. Wollin *et al.*^{12,14} have found that magnetic intensity and temperature are inversely correlated.

One mechanism which has been suggested for explaining these apparent correlations asserts that the lower atmosphere

is affected by energetic particles emanating from the Sun in flares. These particles could change the ionisation balance in the lower atmosphere, affecting among other things its conductivity¹⁰ and ozone content^{10,15}. Access of particles with a given energy is strongly regulated by the Earth's intrinsic magnetic field, as well as the coupling between the solar wind and the magnetosphere. Thus, the correlations cited above could be rationalised according to this picture. Replacing the solar wind by interstellar medium might then have the same effect (a reduction in the flux of solar particles reaching the Earth) as making the Earth's magnetic field very strong. If terrestrial temperature is indeed correlated with energetic particle flux, then shutting off the solar wind may be just what it takes to trigger an ice age.

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Possibility of detecting magnetospheric radio bursts from Uranus and Neptune

EARTH, Jupiter and Saturn are sources of intense but sporadic bursts of electromagnetic radiation which we call magnetospheric radio bursts (MRBs). The Earth's MRBs are observed at kilometric wavelengths, with a power flux spectral peak near 200–300 kHz, a bandwidth roughly half the peak frequency, and a high frequency cutoff near but below the electron gyrofrequency corresponding to the polar surface magnetic field. The Earth's MRBs last a few minutes. Jupiter's MRBs occur at decametric wavelengths, with a power flux spectral peak near 7–8 MHz, a bandwidth again roughly half the peak frequency, and a high frequency cutoff below the polar surface electron gyrofrequency; Jupiter's MRBs also last a few minutes. Saturn's MRBs are observed at hectometric wavelengths, with a power flux spectral peak near 1 MHz and a bandwidth of roughly half the peak frequency, and again last a few minutes. The similarities in the power flux spectra together with the burst occurrence patterns suggest a common physical origin for the MRBs of all three planets. Perhaps the common mechanism is noise amplification by field aligned currents since Gurnett¹ has shown that Earth's MRBs are associated with bright auroral arcs which involve intense field aligned currents. Field aligned currents result from the interaction of the solar wind with the magnetosphere and should be a general feature of the interaction between the solar wind and a planetary magnetosphere (Vasyliunas² and Kennel³). If MRBs are ultimately produced by the solar wind–magnetosphere interaction, their total radiated power might scale as the solar wind power input into the magnetosphere. Kaiser and Stone⁴ have suggested the frequency of emission scales as the polar magnetic field strength of the planet. Here, we scale the intensity of MRBs to the solar wind

Table 1 Values for quantities used in estimating the detectability of MRBs are listed for each of the planets

	B_p (gauss)	B_{Bode} (gauss)	$\frac{P_N b_N}{\dot{W}_p}$	$\dot{W}_p$ (W)	Peak frequency (MHz)	Peak power flux at Earth (W m ⁻² Hz)	Total radiated power (W)
Earth	0.5†	0.5	$\sim 10^{-3}$	5×10^{11}	0.25†	—	5×10^8
Jupiter	14–18†	14	3×10^{-2}	5×10^{13}	8†	$2 \times 10^{-10}†$	1.5×10^{12}
Saturn	2*	4.8	5×10^{-2}	5×10^{12}	1.1†	$5 \times 10^{-20}†$	3×10^{11}
Uranus	—	1.6	$5 \times 10^{-2*}$	4×10^{11}	0.85	10^{-21}	2×10^{10}
Neptune	—	1.3	$5 \times 10^{-2*}$	1.5×10^{11}	0.7	2×10^{-22}	8×10^9

†Measured.

*Inferred from scaling law.

The values of peak frequency, power flux and total radiated power for Uranus and Neptune are calculated assuming the Bode's law value of the polar field strength. The total radiated power is based on the assumption of isotropic hemispherical radiation.

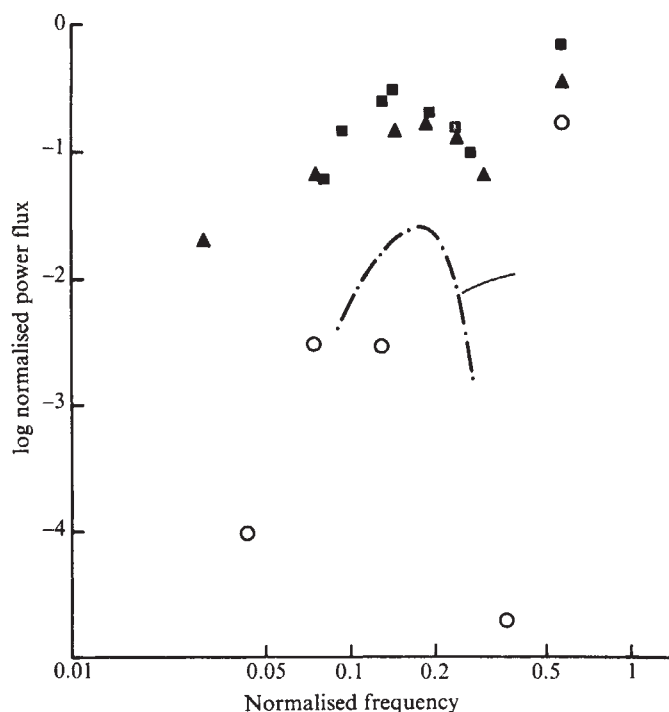
input and the frequency of emission to the polar field strength to estimate the possibility of detecting MRBs from Uranus and Neptune.

For determining the detectability of MRBs, we hope to find a scaling law that relates the ratio of power radiated in MRBs to the solar wind input for Earth, Jupiter and Saturn. The total power radiated in MRBs, P_p , relative to the solar wind dissipation rate, $\dot{W}_p$, can be found by integrating the observed power flux, P_p' , over frequency, ω , and multiplying by the area, A , over which the noise is radiated

$$\frac{P_p}{\dot{W}_p} = \frac{A}{\dot{W}_p} \int P_p' d\omega = \int P_p' \frac{A \Omega_p}{\dot{W}_p \Omega_p} d\omega = \int \bar{P}_p' d\bar{\omega} \quad (1)$$

where $\bar{\omega}$ is the frequency normalised to the electron gyrofrequency corresponding to the surface polar field strength, Ω_p , and $\bar{P}_p'$ is the power flux normalised by the quantity $A \Omega_p / \dot{W}_p$.

Fig. 1 The normalised observed power flux spectra, $\bar{P}_p'$, (see equation (1)) of very intense radio bursts from Earth, ○ (Gurnett¹), Jupiter, ▲, and Saturn, ■ (Brown¹⁰) are plotted as functions of normalised frequency. Saturn's field is predicted by using the magnetic Bode's law. All spectra peak between 10–20% of the surface electron gyrofrequency and have similar bandwidths, but Jupiter and Saturn produce more power relative to the solar wind input than does the Earth. For completeness we have also sketched (— · — · —) a spectrum based on properties of the Earth's MRBs described by Kaiser and Stone⁴, whose experiment had many more channels in the frequency range of interest than did Gurnett's¹, but not enough dynamic range to measure the most intense MRBs of the Earth.



To find the area, A , we assume the radiation is isotropic over a hemisphere. In doing this, we overestimate the total power radiated in MRBs since Earth's MRBs are known to be somewhat beamed (Gurnett¹) while the beaming of Jupiter's high frequency decametric radiation is well known (Warwick⁶). The beaming of radiation at frequencies near the peak power flux is, however, far less pronounced (Dulk and Clark⁶) than at higher frequencies near the electron cyclotron frequency where the power flux is much lower. Since we cannot systematically estimate the beaming factor, we choose to assume isotropic hemispherical radiation for the purpose of scaling.

The solar wind power dissipation can be estimated from the work done by the magnetopause currents at the nose of the magnetosphere in the motional e.m.f. of the solar wind. Using standard theory to define solar wind parameters, Kennel⁷ estimated that the power dissipation $\dot{W}_p$ into a given planetary magnetosphere scales to that at earth $\dot{W}_E$ as

$$\frac{\dot{W}_p}{\dot{W}_E} = \left(\frac{M_p}{M_E} \right)^{2/3} r^{-4/3} = \left(\frac{R}{R_E} \right)^2 \left(\frac{B_p}{B_E} \right)^{2/3} r^{-4/3} \quad (2)$$

where $M = B_p R^3$ is the magnetic dipole moment, B_p is the surface magnetic field, R is the planet's radius, and r its heliocentric distance in AU.

Kaiser and Stone⁴ point out that Jupiter's observed polar field strength can be correctly predicted from the observed properties of the MRBs of Earth and Jupiter by assuming that the maximum (cutoff) frequencies of MRBs are the surface electron gyrofrequencies and that the maximum and peak frequencies of MRBs scale linearly with B_p . Using the observed properties of SMRB and the same assumptions they estimate a polar field strength of 2 gauss for Saturn. Saturn's estimated field strength also agrees within a factor 2 of the 'magnetic Bode's law'⁸ estimate (used by many authors as a guide to estimate the magnetic fields of planets where observations were not available), which assumes that a planet's dipole moment M scales as its rotational angular momentum L . While there is little theoretical justification for the magnetic Bode's law, Hill and Michel⁹ have pointed out that it is surprisingly well obeyed by the objects in the Solar System, in the sense that the spread in M/L is much less than the spread in L .

Using measured values of B_E and B_p and the Bode's law estimate for B_s , we plot in Fig. 1 the normalised power flux spectra $\bar{P}_p'$ of the MRBs of Jupiter and Saturn measured by Brown¹⁰ and a spectrum of Earth's MRBs measured by Gurnett¹. We can approximate the integration of the power flux in equation (1) by taking the peak normalised power flux, P_N , and multiplying by b_N , the bandwidth in normalised frequency

$$P_p / \dot{W}_p \approx P_N b_N \quad (3)$$

Using equation (3), we see from Fig. 1 that Jupiter and Saturn may radiate 1–5% of the solar wind energy input into the magnetosphere while Earth may radiate only ~0.1%. These percentages must be viewed with caution, however, since the Earth's MRBs shown may not represent the most intense

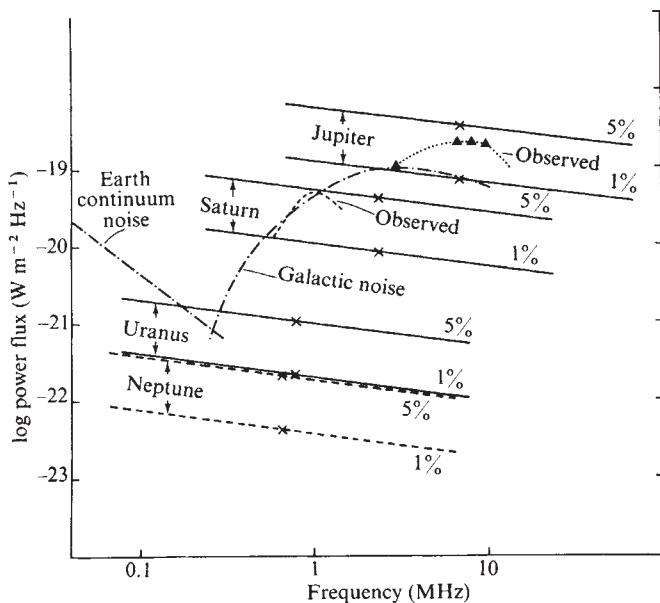


Fig. 2 The locus of the peak in the MRB power flux spectrum at Earth is shown as a function of surface magnetic field strength for efficiencies of 1 and 5% conversion of solar wind power input into the planet's magnetosphere to the most intense MRBs. The surface field strength ranges from 0.1 to 10 times the magnetic Bode's law value, which is indicated by a cross for each planet. The peak frequency is assumed to vary linearly with surface field strength, B_p . The bandwidth of MRBs is taken as half the peak frequency, and since the solar wind power input varies as $B_p^{2/3}$, the peak power varies as $B_p^{-1/3}$. The peak power flux of MRBs from Uranus and Neptune is conjectured to lie between the 1–5% lines. The noise level of a short electric dipole in Earth orbit at $30R_E$ is indicated by ---. Both Uranus and Neptune are near the noise levels, but MRBs from both planets may be detectable from Earth orbit, especially if the surface field strengths are below the magnetic Bode's law estimate.

events; the total number of observed Saturn's MRBs is small, and the MRB spectrum of Jupiter shown is a composite obtained by averaging the intensities of the most intense events. The intensity will also depend on the beaming of the radiation and may further be affected by factors we have not discussed, such as Io-like moons inside the magnetosphere. The close similarity of the MRB normalised spectra of Jupiter and Saturn, and the similar, but lower, MRB normalised spectra of the Earth, suggest, however, that the scaling of MRB power to the solar wind-magnetosphere dissipation power is a reasonable hypothesis.

We now estimate the properties of MRBs from Uranus and Neptune by assuming a 1–5% conversion efficiency, $W_E = 5 \times 10^{11}$ W, a bandwidth of half the peak frequency, and that dipole moments of Uranus and Neptune roughly obey the magnetic Bode's law. In Fig. 2 we plot the peak power flux for 1 and 5% efficiency for Uranus, Neptune, Saturn and Jupiter, assuming various values of B_p . The Bode's law estimates are indicated by a cross. We have superposed curves of Earth interference at $30R_E$ and galactic noise for a short dipole antenna. In Table 1 we list the parameters used in estimating the detectability of MRBs.

The noise curves probably give a too pessimistic impression concerning the detection of MRBs for Uranus and Neptune, since knowing the location of a possible bursty source and using an antenna more directional than a short dipole may facilitate detection below the noise level. Figure 2 also indicates that the situation only improves away from Earth, since the Earth continuum noise is reduced and the power fluxes from Uranus and Neptune should increase as the square of the distance to the planet. We therefore suggest that detection of magnetospheric radio bursts from Uranus and Neptune might be a reasonable cruise mode radio astronomy objective on future missions to the outer Solar System.

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Dirac's continuous creation cosmology and the temperature of the Earth

DIRAC^{1,2} has modified his earlier cosmological model based on the large number hypothesis^{3,4}, that the observed approximate equalities

$$\frac{e^2}{Gm^2} \approx \frac{mc^3}{He^2} \approx \left(\frac{\rho c^3}{mH^3} \right)^{\frac{1}{2}} \approx 10^{39} \quad (1)$$

are not accidental but causal—even though we are as yet unaware of the nature of the relationship between cosmology and local physics (e is the electronic charge, G the gravitational constant, c the velocity of light, ρ the mean density of the Universe, H Hubble's constant and m an atomic mass). In the earlier version, Dirac assumed mass conservation and that equalities (1) held for all time, thus

$$R(t) \propto t^{1/3}; H \propto \frac{1}{3t}; G \propto \frac{1}{t} \quad (2)$$

where $R(t)$ is the cosmological scale factor, $H = \dot{R}/R$. In the modified version, Dirac argues (unconvincingly in my view) that $R(t) \propto t$, in which case

$$G \propto \frac{1}{t}; H \propto \frac{1}{t}; \rho = \frac{\rho_0}{t^3} \propto \frac{1}{t^3}; \rho_0 \propto t^2$$

that is, matter is continuously created such that $\rho_0 \propto t^2$ where t is the age of the Universe. This newly created matter could be produced uniformly throughout space, or locally in proportion to the amount and composition of matter already present. Dirac prefers the second alternative, but we shall show that this leads to an unacceptable past temperature for the Earth.

What are the properties of the newly created matter; is it at rest relative to the cosmological background, or is it created at rest relative to the local matter? If the creation field is a scalar we would expect the former, but it could be a vector or tensor field and lead to the latter. (I would like to thank the referee for emphasising this second possibility.) In the absence of any way of deciding between these two possibilities we consider both; in either case the mass of any body, $M \propto t^2$.

The luminosity of a star of solar mass satisfies the approximate proportionality

$$L \propto G^7 M^5 \propto t^3 \quad (3)$$

and increases with time. On the other hand the orbit of the Earth with mass $m(t)$ satisfies the equation

$$\frac{d}{dt}(m\dot{r}) = -\frac{GMm}{r^2} \hat{r} \text{ (creation at rest)} \quad (4)$$

or

$$m \frac{d\dot{r}}{dt} = -\frac{GMm}{r^2} \hat{r} \text{ (comoving creation)} \quad (4a)$$

These equations have an angular momentum integral

$$mr^2\dot{\theta} = A \text{ (a constant)} \quad (5)$$

or

$$r^2\dot{\theta} = h \text{ (a constant)} \quad (5a)$$

and therefore the reciprocal radius, u , satisfies the orbit equation

$$\frac{d^2u}{d\theta^2} + u = \frac{GMm^2}{A^2} \quad (6)$$

or

$$\frac{d^2u}{d\theta^2} + u = \frac{GM}{h^2} \quad (6a)$$

Thus the radius R of the orbit varies in time

$$R \propto \frac{A^2}{GMm^2} \propto t^5 \quad (7)$$

or

$$R \propto \frac{h^2}{GM} \propto t \quad (7a)$$

The orbit spirals inwards in both cases. Combining equations (3) and (7) (or (7a)) we find the equilibrium temperature of the Earth

$$T_E \propto \left(\frac{L}{R^2}\right)^{1/4} \propto (G^9 M^{11})^{1/4} \propto t^{13/4} \quad (8)$$

or

$$\propto (G^9 M^7)^{1/4} \propto t^{5/4} \quad (8a)$$

Now the inverse Hubble constant has a value of about 2×10^{10} yr which in this cosmological model $R(t) \propto t$ is the age of the Universe; since the Earth has an age of $\sim t_E = 5 \times 10^9$ yr, the temperature has increased from

118 K ($t_E = 0$) to 177 K ($t_E = 3 \times 10^9$ yr) to 300 K
(creation at rest)

209 K ($t_E = 0$) to 245 K ($t_E = 3 \times 10^9$ yr) to 300 K
(comoving creation)

The Earth was substantially cooler in the past than today. If our understanding of climatology is at all correct, this would imply that the Earth was covered with ice and such an ice cover would not have melted even when the equilibrium temperature reached its present value, because of the high albedo of an ice-

covered Earth. The existence of life some 3×10^9 yr ago would apparently rule out these cosmological models.

Uniform creation would leave the masses of the Sun and Earth effectively constant so that with $G \propto 1/t$

$$T_E \propto \left[\frac{1}{t}\right]^{9/4} \quad (9)$$

Since in this model $R(t) \propto t$, $H = 1/t$, the age of the Universe is 2×10^{10} yr, and from equation (9) it follows, therefore, that some 3×10^9 yr ago the temperature at the Earth would have been

$$T_E = \left(\frac{20}{17}\right)^{9/4} 300 \text{ K} = 412 \text{ K} \quad (10)$$

too hot for life to have developed, unless the increased solar flux does not lead to a corresponding increase in the Earth's temperature because of the increased albedo of a cloud-covered Earth.

A way out of this problem is to have

$$T_E \propto (G^9 M^{11})^{1/4} \quad (11)$$

a slowly varying function of time; for example, since equation (1) gives $G \propto 1/t$, if we take $M \propto t$ then $T_E \propto t^{1/2}$, slightly cooler in the past but not uncomfortably so. This then requires $\rho_0 \propto t$ and thus

$$R(t) \propto t^{2/3} \quad (12)$$

the Einstein-de Sitter cosmological solution, only now there is creation of matter proportional to the age of the Universe. For such a model the age of the Universe is $2/3H$ or 1.4×10^{10} yr so that 3×10^9 yr ago, the Earth's temperature would be $\sim 15\%$ cooler, or 45 K below the present value, which is within the error of uncertainty of climatic models.

While such a model, or a similar one, may not be inconsistent with the observed data, it is somewhat *ad hoc*.

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Variable G : a solution to the missing mass problem

DIRAC¹ suggested in 1937 that the gravitational 'constant' G might vary with time. This idea has since been used in several cosmologies, particularly those of Hoyle and Narlikar² and those invoking the Brans-Dicke theory of gravity³. Limits to $\dot{G}$ have been suggested by Barnothy and Tinsley⁴ from the observed form of the redshift-magnitude relation and by Dearborn and Schramm⁵ from the existence of clusters. Nevertheless, Van Flandern⁶ has provided substantial observational support for a secular decrease in G such that $\dot{G}/G = -(7.5 \pm 2.7) \times 10^{-11}$

yr⁻¹, and this is supported by Morrison and Ward's⁷ work on the transits of Mercury. The idea deserves to be explored further, pragmatically in non-cosmological contexts, but we are here primarily concerned with the explanation it affords for the 'missing mass' paradox.

Let us begin with the orbits of two point masses. It is easy to verify numerically Vinti's^{6,8} relationships

$$\frac{\dot{G}}{G} = -\frac{\dot{a}}{a} = \frac{\dot{n}}{2n} = -\frac{\dot{T}}{2T}$$

for secular changes in the semi-major axis a , period T and mean angular motion n of an orbit with $T \approx 10^8$ – 10^9 yr. We use a fourth order Runge-Kutta⁹ algorithm to solve the equations of motion in polar coordinates (r, θ) for a field $G \propto t^{-1}$. For all times after the epoch of unbinding, as judged from the total energy $E \equiv 0$, the radial expansion $r \approx -r\dot{G}/G$. With Van Flandern's value for $\dot{G}/G$, this relationship gives an expansion velocity of 73 km s⁻¹ Mpc⁻¹ to components of erstwhile bound systems. This formulation and the value of $\dot{G}/G$ thus parallel the Hubble Law and the value of the Hubble constant, H . They may be identical.

When G varies with time it is necessary to consider the radial expansion component within all large scale associations of self-gravitating objects. Emphasis need no longer be placed on the usual limitation of the cosmological expansion, to the regions between groups and clusters of galaxies. The average mass of a galaxy is usually estimated from the internal motions of many objects or by application of the virial theorem to the velocities and separations found within rich clusters. These methods produce conflicting results¹⁰, and typically $10 < \beta < 400$ where β is the ratio of the virial estimate to the mass identified by all other techniques. Expansion has a negligible effect on the dynamics of ordinary galaxies, which for $r < 30$ kpc have $r \lesssim 2$ km s⁻¹. The stability of groups and clusters can now be considered.

The orbits of 2 galaxies with a combined mass of $2.5 \times 10^{11} M_\odot$ were simulated using $\dot{G}(t) = G_0 (1.5 \times 10^{10} t)^{-1}$, where G_0 is the present value, when initially circular orbits become unbound $r \approx 2r_0$ and

$$r = r_{lim} \approx 0.8 \left[\frac{8MG}{5} \left(\frac{G}{\dot{G}} \right)^2 \right]^{1/3}$$

Orbits with large eccentricity may be unbound at $\sim r_{lim}/2$. The simulated orbits show that now, for comparatively large galaxies, $r_{lim} \approx 580$ kpc, in agreement with Holmberg's¹¹ observational result ($H = 55$ km s⁻¹ Mpc⁻¹). Those initially circular orbits that now have a separation of ~ 1.5 Mpc simulate the properties of many small groups. These orbits have $r \approx 3r_0$, so the observed r.m.s. velocity dispersion $\langle V^2 \rangle_{obs} \approx 10 \langle (r\dot{\theta})^2 \rangle$, and the virial mass is overestimated by a factor of ten. Small groups still persist because such orbits exist and have been unbound for only 10^{10} yr, and because $\langle V^2 \rangle_{obs}$ is a function of the characteristic size, R , of the group.

Estimates of the virial mass of the small groups are very inexact because $\langle V^2 \rangle_{corr}$ is the difference of two large quantities and is very sensitive to inaccuracy in either, and because it depends on the precise value of $\dot{G}/G$ and on the implicit assumption of an isotropic distribution of circular orbits. If the line of sight expansion component is calculated from

$$V_R^2 = \frac{1}{3} \left(\frac{\pi}{2} R \dot{G} G^{-1} \right)^2$$

18 of the 50 groups with data listed by Rood, Rothman and Turnrose¹⁰ have negative velocity dispersions, and the remaining data show a residual dependence of R on V . Thus with the Sculptor Group of galaxies¹², which have well determined

21-cm velocities for six of the seven core 'members' and independent estimates for most masses

$$\langle V^2 \rangle_{corr} \approx 107^2 - 79.5^2 = 71.6^2 \text{ and } R = 1.2 \text{ Mpc}$$

using data weighted by masses that are calculated assuming a common distance of 3.0 Mpc. When the data are weighted in proportion to the apparent luminosities, however,

$$R = 1.6 \text{ Mpc}$$

and

$$\langle V^2 \rangle_{corr} = 100^2 - 105^2$$

Before the virial theorem can be applied it is necessary to determine an independent distance to every member. Those members receding almost in the line of sight have the smallest apparent separations from the group centre and are likely to be both the faintest objects and to have the largest recession velocities and conversely for approaching objects. Besides explaining the effect noticed by Arp¹³, this process significantly biases the estimates of R and V .

The masses usually quoted for the Coma cluster refer to the well studied region with $r \lesssim 6^\circ$. Using data from Rood *et al.*¹⁴, $R_c = 3.7$ Mpc, $\langle V^2 \rangle_{corr} = 861^2 - V_R^2 = 817^2$ so the adjusted mass from Poveda's¹⁵ method is $M = 9 \langle V^2 \rangle_{corr} R_c G^{-1} = 3.0 \times 10^{15} M_\odot$ with $\beta \approx 4$, and $M/L \approx 120$. But when the outlying 'members' at radii of 14.2° (32 Mpc) are included²², the lower limit to the mass is $1.3 \times 10^{16} M_\odot$ with $\beta \approx 16$. This limit is inappropriate when G varies. Thus three particular orbits that result now in a separation of 32 Mpc from an initial orbit with eccentricity e at an epoch of 1.5×10^9 yr require

$$\begin{aligned} M_{Coma} &= 2.0 \times 10^{14} M_\odot \text{ (if } e = 0.9, \text{ start pericentre)} \\ &= 1.2 \times 10^{15} M_\odot \text{ (} e = 0.0 \text{)} \\ &= 2.3 \times 10^{16} M_\odot \text{ (} e = 0.9, \text{ start apocentre)} \end{aligned}$$

These are all an order of magnitude smaller than the above limit, and there is clearly sufficient flexibility in the range of possible orbits, to ensure the existence of solutions with $\beta \approx 1$.

An extension to systems with extended mass distributions is possible with models such as King's¹⁶ models of elliptical galaxies, which relate the mass to a core radius r_c through

$$M = 9\mu r_c \sigma_V^2 (4\pi G)^{-1}$$

Here μ is a constant determined by the value of r_{tidal}/r_c adopted and r_{tidal} is the limiting radius. For NGC3379, King finds $r_{tidal} \approx 160r_c$ so that any increase in mass for a model of given velocity dispersion σ_V is associated with a large increase in r_{tidal} . The mass has an upper limit when $r_{tidal} = r_{lim}$. For a brightest cluster-elliptical such as NGC4486 (ref. 17), $\sigma_V = 550$ km s⁻¹, $r_{lim} < 4.7$ Mpc and $M < 10^{14} M_\odot$. When allowance is made for the predominantly radial orbits to be expected in the outer regions, these limits reduce to $M < 4 \times 10^{13} M_\odot$ and $r_{lim} < 1.6$ Mpc. More self-consistent models should reduce this to $\sim 10^{12} M_\odot$. The variability of G here explains in principle both the observed small range in the absolute magnitudes of the brightest cluster-elliptical and its independence of the size and richness of the cluster¹⁸.

The hypothesis of a variable gravitational constant can completely solve the 'missing mass' problem, and promises to assist the study of the formation, structure and evolution of galaxies. Already it is evident that Lynden-Bell's¹⁹ violent relaxation method will be greatly facilitated. Most galaxies probably form by the fragmentation of a protocluster gas cloud, following processes like those described by Reddish²⁰. With a steadily weakening gravitational field, the subsequent breakup of most early clusters into small groups and isolated galaxies is to be expected, and predicts the quasi-homogeneous distribution of single galaxies found by Turner and Gott²¹.

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The Madagascar controversy still lives

WE feel that the title and conclusions of the report by P. J. Smith¹ of new palaeomagnetic data from Madagascar² does not reflect an objective assessment of the arguments for the palaeo-position of Madagascar. In particular your correspondent considers that any geological evidence against a northerly derivation must now be considered anomalous and that the new palaeomagnetic information provides incontrovertible evidence for the movement of Madagascar from the East African coast in post-Lower Jurassic times. We would suggest that the Madagascar issue, which is critical in defining the fit of eastern and western Gondwanaland³, is far from settled and that the new 'modern' data must be examined and evaluated against all other evidence: geological, geophysical and geochemical.

There seems to be a misunderstanding of the fundamental evidence against a northerly derivation of this micro-continent. The argument does not depend on the matching of coastlines, marine incursions and so forth, as such arguments are rarely diagnostic. In this particular case, the presence of Jurassic marine sediments in the Knysna outline in South Africa⁴ suggests the presence of a Jurassic coastline along most of the Indian Ocean coast so that matches can be made in various localities (although the coincidence of late Jurassic-early Cretaceous volcanics on previously similar latitudes may be significant). The fundamental geological argument hinges on the existence in Kenya-Tanzania of thick sediments on-shore, and for considerable distances off-shore, that were deposited from early Karroo times (Upper Carboniferous) onwards. These sediments have been shown in numerous drillings and have been traced for several 100 km into the Indian Ocean basin by seismic profiles⁵⁻⁷. There seems to be no possibility that these deposits could exist in their present position if this was occupied by a micro-continent that has since moved away. Subsidiary indications against such a northerly fit are also indicated by the seafloor anomalies of the Indian Ocean⁸ which show Madagascar in its present position some 75 Myr ago, at a time when India, which is placed against Madagascar in most reconstructions, was already level with or slightly south of Madagascar.

Samples from seven of the nine sampled sites in the Isalo Group of Madagascar have been thoroughly and carefully

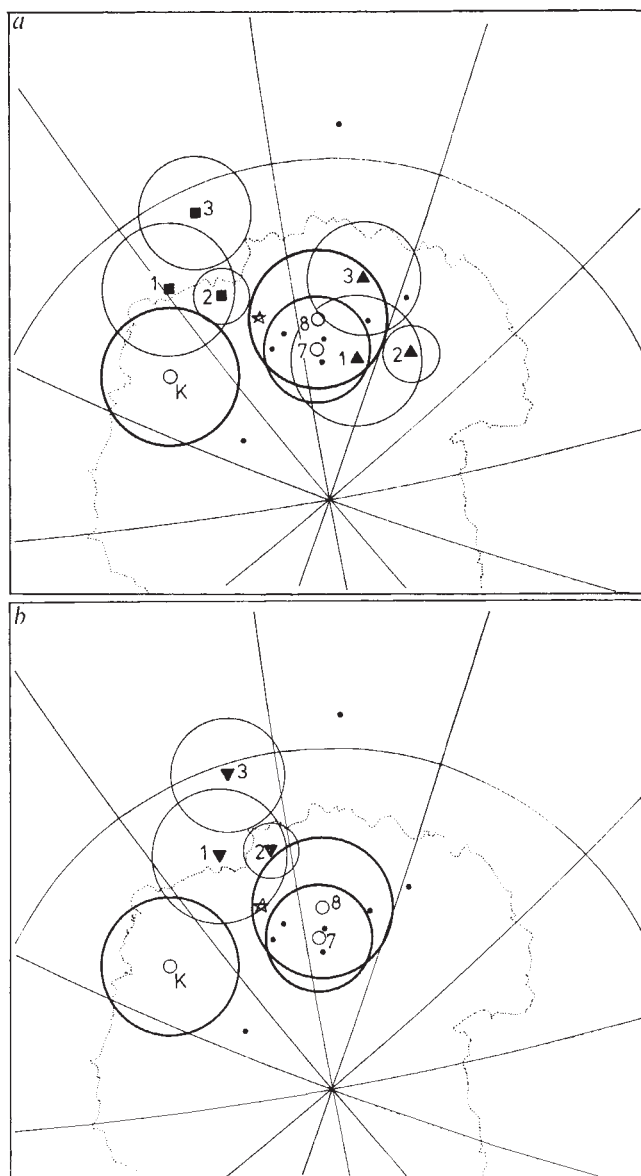


Fig. 1 Madagascan and African pole positions. Site poles from the Isalo Group (Triassic-Jurassic) of Madagascar are shown as dots, with the mean poles and associated circles of 95% confidence using all stable sites (8) and all well grouped sites (7). The mean pole position for Madagascar Cretaceous sites is also shown (K). The virtual geomagnetic pole corresponding to the present field direction in Madagascar is shown as a star. 1, 2 and 3 correspond respectively to the mean African Triassic-Jurassic pole used by Embleton and McElhinny², the Triassic-Jurassic site-mean pole from southern Africa and the Cretaceous site-mean pole for southern Africa. *a*, The Madagascar data are shown relative to the African data expected according to the Smith and Hallam reconstruction (▲) and the Flores position^{16,17} (■) using the rotation suggested by Embleton and McElhinny². *b*, The Madagascar data in relation to African poles (▼) assumed no differential movement between them during Mesozoic and Tertiary times. All circles are of 95% confidence.

studied² and these palaeomagnetic observations, on their face value, are statistically consistent with only a northerly derivation of Madagascar. In view of the incompatibility of the palaeomagnetic and geological data, it is necessary to assess the possible sources of error in the palaeomagnetic data which can arise in excess of the statistical estimates. The sediments involved are continental sandstones and mudstones which, in modern environments, undergo extensive oxidation and haematization over periods of many hundreds of thousands of years^{9,10}. The within-site scatter of this limited collection is reported as being small² and to have been acquired over some time. The magnetisation is

not, therefore, of detrital nature, but is caused by chemical changes occurring after deposition. It is extremely difficult to isolate the magnetisation associated with detrital particles from that associated with later diagenetic changes¹¹, particularly as the detrital grains themselves may be changed during diagenesis and such haematisation of continental sediments may be delayed for at least 100 Myr, as in some Siluro-Devonian sediments¹²⁻¹⁴. It seems entirely possible, therefore, that the observed remanence is complex and that thermal and alternating magnetic field demagnetisation may not have effectively separated the different magnetic components, as has been suggested by Storetvedt¹⁵ in connection with many continental sediments. It may be no coincidence that the pole corresponding to the present geomagnetic field in Madagascar (star on Fig. 1) is at least as consistent with the Isalo palaeomagnetic data as is the mean Triassic-Jurassic pole used by Embleton and McElhinny⁷.

Even if the magnetisation is of similar age to the rocks, there are various ways in which comparison with other data of the same age can be made. Embleton and McElhinny⁷ used data from all of Africa, assuming no differential movement between, for example, Sicily and South Africa, and also gave equal weight to each formation mean pole position. If only southern Africa data are considered, which are extensive and numerous, and the same weighting is used as in the analysis of the Madagascar data, that is, equal weighting to each site, then the result is significantly different from that of Embleton and McElhinny (Fig. 1). If anything, there is better agreement between the southern African pole (2) and the Madagascan pole (8) with both areas in their present positions in Triassic times. If the more scattered Isalo sites are omitted, then the resultant pole (7) differs almost equally from all three postulated positions. Smith¹ also ignores the Lower Cretaceous palaeomagnetic data from Madagascar¹⁸⁻²⁰ which are not consistent with postulated southern African Upper Cretaceous data, although furthest from the position expected if Madagascar was then in its northerly position or moving from it. We would not claim that our statistical analysis is superior or inferior to that used by Embleton and McElhinny, but the fact that different interpretations could be 'proved' by such analyses indicates that the quoted errors exceed the real errors involved in the comparisons.

The new palaeomagnetic data are therefore welcome additions to the available information relevant to the derivation of Madagascar, but there are clearly more grounds for considering the interpretations^{1,2} of these as 'anomalous' than the vast range of geological and geophysical studies that are inconsistent with a northerly derivation. Nonetheless, we would prefer that the controversy remains open for further testing and consider that it is premature, and unscientific, to allow an uncritical acceptance of one line of evidence to suggest that further investigations are redundant.

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Grain-boundary migration in metals fatigued at high temperatures

THE migration of grain boundaries in polycrystalline metals fatigued at high homologous temperatures has been reported for FCC, BCC and CPH metals¹⁻⁴. Migration results in a 'diamond' or orthogonal configuration of boundaries with segments aligned in the maximum shear stress directions. It has been suggested that this migration contributes to grain-boundary failure by the absorption of point defects at migrating boundaries⁵ and/or by the development of the diamond configuration of boundaries, which puts these boundaries in a favourable arrangement for sliding¹⁻⁴. In contrast to the information on cavity growth during high temperature deformation, there seems to be little quantitative information on grain-boundary migration in these conditions. We report here the results of measurements of grain-boundary migration on Al, Cu, Zr and Zircaloy-2 specimens fatigued at temperatures where grain-boundary cavitation occurs. These results indicate that in the initial 10% of the fatigue life the rate of boundary migration decreases with time and the amount of migration is $\propto \text{time}^{2/3}$. The possible influence of boundary migration on cavitation is suggested to be limited to the initial part of the fatigue life.

Table 1 gives the details of heat treatment, composition, grain size and test temperatures of specimens fabricated from sheet 1 mm thick. Specimens were chemically polished before testing in the reverse plane-bending mode of fatigue in high vacuum conditions (air pressure $< 1.33 \times 10^{-4}$ Pa) at 16 Hz. The test conditions were typical of low strain-high cycle conditions, that is, the strain amplitude was in the range ± 0.06 – $\pm 0.257\%$ which gave fatigue lives $> 10^6$ cycles. At various stages of the fatigue life, the cyclic straining was stopped and the specimen allowed to cool to room temperature

Fig. 1 Log (average grain-boundary migration) against log (number of cycles) for: $\square$, Al at 260 °C and a strain amplitude of $\pm 0.073\%$; $\diamond$, Cu at 500 °C and a strain amplitude of $\pm 0.11\%$; $\circ$, Zr (crystal bar) at 700 °C and a strain amplitude of $\pm 0.125\%$; $\times$, Zr (crystal bar) at 700 °C and a strain amplitude of $\pm 0.21\%$; ∇ , Zircaloy-2 at 700 °C and a strain amplitude of $\pm 0.21\%$. The length of the vertical bars indicates the $\pm$ s.e. of the points. 'F' indicates failure.

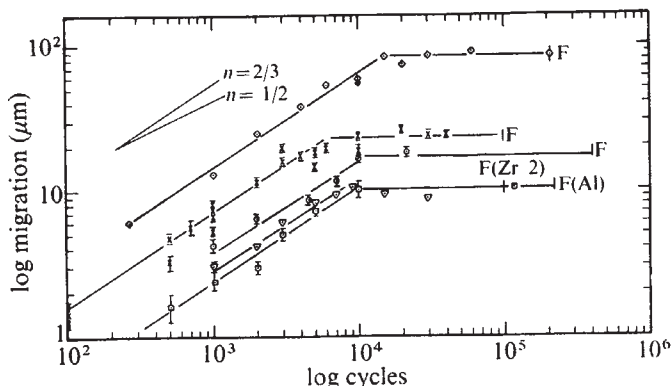


Table 1 Details of experimental technique

Metal	Test temp (°C)	Heat treatment (h at °C)	Purity	Grain size (µm)
Al	260	3 at 460	0.004 weight % total impurity	85
Cu	500	1 at 600	Commercial grade 0.014% O ₂ ; 0.002% Mg; <0.05% As; <0.01% P; <0.01% Si	35
Zr	700	3 at 750	Crystal bar 0.00035 weight % O ₂	60
Zircaloy-2	700	50 at 850	Standard commercial alloy 1.4% Sn; 0.14% Fe; 0.1% Cr; 0.05% Ni	60

under vacuum. The specimen was removed from the machine and examined under the microscope at a magnification of $\times 500$ using a calibrated eyepiece. Grain-boundary migration was confined to the gauge length of the specimens.

In the case of Al, Zr and Zircaloy-2, the average migration distance, $\bar{m}$, was less than half the initial grain size and $\bar{m}$ was obtained from the widths of the migration traces left by at least 100 randomly selected grain boundaries⁶. For Cu, $\bar{m}$ was so large that traces left by migrating boundaries often covered whole grains, so that it was impossible to associate the traces with a particular boundary or a set of boundaries. In this case, $\bar{m}$ was estimated from $\bar{m} \approx (\bar{D} - D_0)/2$ where D_0 is the initial grain size and $\bar{D}$ is the average grain size at a particular stage of fatigue testing. After repolishing the specimen, the grain size was measured using the linear intercept method.

The average rate of grain-boundary migration was found to decrease with increasing N and, after $\sim 10^4$ cycles or $\sim 10\%$ of the fatigue life, approached a limiting rate close to zero. Values of $\bar{m}$ of up to 80 µm were obtained. Typical results are shown in Fig. 1 in which $\log \bar{m}$ is plotted against $\log$ of the number of strain cycles, N . The initial part of the $\log \bar{m}$ against $\log N$ curves for each of the materials can be approximated by straight lines of slope 2/3. The initial part of these curves can be represented by

$$\bar{m} \propto N^n \propto t^n$$

where n is a constant whose value is close to 2/3 and t is the test time. A least squares fit to 94 average values of $\bar{m}$ (representing over 9,400 individual measurements of boundary migration) during the initial stage of fatigue testing of Al, Cu, Zr and Zircaloy-2 gave a value of $n = 0.653$ with a s.d. ± 0.070 .

Figure 1 also shows that the magnitude of $\bar{m}$ was dependent on both strain amplitude and material, whereas the initial slope, n , was independent of these factors. The value of n ($\approx 2/3$) is greater than the upper limiting value of $n = 1/2$ for metals undergoing normal grain growth^{7,9} or a value of $n = 0.31$ for acoustically assisted grain growth in Cu⁹. Comparison of this value of n with that for grain growth is, however, not strictly valid because of the different experimental conditions involved in each technique. The value of n obtained in our work for migration is in the range of the time exponents for cavity growth in Cu fatigued at high temperatures obtained by Gittins⁵. It should be noted, however, that the value of $n = 2/3$ for migration obtained in the present work applies only up to 10% of the fatigue life, whereas the exponent of 2/3 or 1 for cavity growth applies throughout the whole fatigue life. This suggests that in the present experiments, the absorption of point defects by migrating grain boundaries and their effect on cavity growth is only possible up to 10% of the fatigue life. This does not seem to have been considered in the migrating boundary model of cavity growth⁵.

The suppression of grain-boundary migration at this stage of the fatigue test may be explained by three possible mechanisms: (1) A balance may be struck in the defect concentration across boundaries¹. This may occur when the cyclic hardening saturates.

(2) The rapid nucleation and growth of grain-boundary cavities during the first 60 s of fatigue testing, reported by Gittins⁵, suggests that the presence of grain-boundary cavities would exert a significant drag on migrating boundaries¹⁰. The

cavities may eventually grow to a size where they could prevent grain-boundary migration.

(3) The formation of the 'diamond' grain structure and the enhanced grain-boundary sliding that is possible for boundaries in this configuration may limit grain-boundary migration. At later stages of the fatigue life, where significant migration no longer occurs, the continued nucleation and growth of cavities would have to depend on other mechanisms such as vacancy capture or grain-boundary sliding. The applicability of these mechanisms is being examined.

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Extinction-free measurements in crystallography

OVER the past fifty or more years extinction has remained a major experimental problem in crystallography¹⁻³. Many strategies have been devised to reduce its magnitude for the accurate determination of physically-significant structure factors (F). Its elimination, as a practical possibility, has appeared remote because extinction is a concomitant of the production of diffraction intensity in the standard techniques. Phenomenologically, extinction is a manifestation of multiple diffraction (see ref. 4), and this being so, an obvious conclusion is that the experimental disposition to be sought is one where the recorded intensity must arise solely from single-scattering events⁵⁻⁷. Then, by definition, the diffracted beam is not 'extinguished' and simple kinematical theory is applicable. Starting with the design of a 'defocusing' X-ray monochromator⁸, I have been investigating the intensity of asymmetric reflections from extended-face crystals over the regions of both positive and negative asymmetry, first with an abraded surface⁹ and then with an optically flat one. The results from this latter study, with an extrapolation of the work of Hirsch and Ramachandran⁵, show that it is feasible to establish values of integrated intensity which involve only single-scattering events.

If one considers an asymmetric crystal in the 'defocusing' mode⁸ (Fig. 1a) the incident beam enters the surface at a shallow angle. When diffraction occurs, the diffracted beam emerges through the surface by the shortest path consistent with the Bragg condition, and this gives the minimal possibility for the diffracted beam to interact with another part of the crystal and thus of energy to be removed by successive diffraction. As one approaches the limit of tilt, $\alpha = -\theta$, the range of penetration below the surface and the possibility of subsequent diffraction events decreases still further. Eventually only single

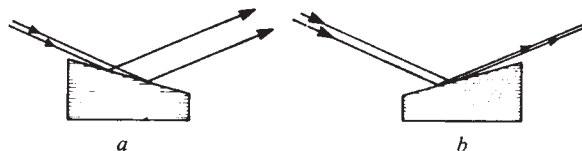


Fig. 1 Asymmetric reflection from an extended-face crystal (a) in the 'defocusing' mode with α negative and (b) in the 'concentrating' mode with α positive.

scattering events contribute to the emergent beam. The measured integrated intensity increases as $\alpha \rightarrow -\theta$, reaching a limit equal to twice the theoretical kinematical value at the symmetrical position $\alpha = 0^\circ$, $\rho_0'(0)$ (refs 10 and 5). For the alternative 'condensing' or 'concentrating' mode (Fig. 1b), any diffraction processes beyond single scattering produce beams directed initially down into the crystal matrix. With increasing tilt, this trend becomes greater until, in the limit $\alpha = +\theta$, only single-scattering events contribute to the emergent beam. This disposition therefore acts as a filter to exclude multiple diffraction events. Near the limit, the emergent intensity tends to zero but its specific intensity tends to twice the corresponding kinematical value at the symmetrical position, $\alpha = 0^\circ$ (ref. 5).

The significance of the optical finish of the surface for (1) the physical requirements of satisfactory extrapolations to $\alpha = \pm\theta$ and (2) ensuring minimum interference of counteractive absorptive effects from surface layers⁹, including protuberances⁸, is evident from the physical picture outlined above.

In terms of integrated intensity, the theoretical values range from the maximum of 2 at $\alpha = -\theta$ to 0 at $\alpha = +\theta$. If the measurements are normalised to effective equal scattering volume, as in Mathieson⁹, the limiting values of $\rho(\beta)(1-\beta)^{-1}/\rho_0'(0)$ at both ends ($\beta = \pm 1$) of the range of the asymmetry β (defined as $\cot \theta \tan \alpha$) are now unity. One may then recognise more readily the symmetrical relationship of the positive and negative tilt regions, in respect of both theoretical (Fig. 2) and experimental curves. In the latter case, the confirmation of symmetry is a valuable diagnostic.

The description above gives a physical interpretation of the theoretical curves presented by Hirsch and Ramachandran for the ratio of integrated intensity (and also specific intensity) of a

perfect crystal with various levels of primary extinction, to the integrated intensity (and specific intensity) of an ideally-imperfect (mosaic) crystal of the same structure. These ratios, plotted against the asymmetry β , are shown in Fig. 2 of their paper⁵. Transformation of those curves for perfect crystals to a normalised basis is shown in Fig. 2 here.

In ref. 5, however, the role of secondary extinction was not included in the theoretical model of the mosaic crystal. With secondary extinction included, the results for the mosaic crystal cannot be represented by the one straight line, (a) in Fig. 2. For real crystals there would be a family of curves dependent upon the magnitude of the secondary extinction, as in the case of primary extinction. The precise shape of curve would differ in detail from that for primary extinction but the relevant point is that the family of normalised secondary extinction curves, plotted as $\rho_s'(\beta)(1-\beta)^{-1}/\rho_0'(0)$ against β , would also converge to a value of unity at $\beta = \pm 1$.

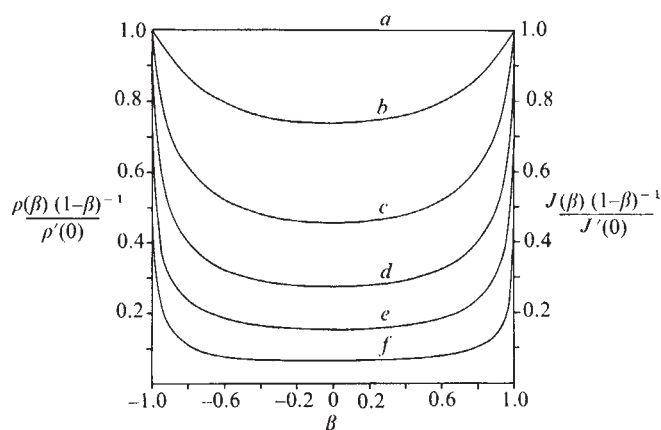
For secondary as well as primary extinction, therefore, extrapolation of normalised values of integrated intensity to $\beta = -1$ and $\beta = +1$ yields numerical values of the integrated intensity which are extinction-free. Because they arise only from single scattering, the values can be equated to the simple kinematical formula and, in these circumstances, the necessary conversion factors, such as for the polarisation component, and the functional dependence on F^2 , are well defined. Further, because the experiment involves the total incident beam, it is relatively straightforward to place the measurements on an absolute basis. To summarise—the real potential of the method is to establish absolute values of structure factors experimentally.

Note that Fig. 2 (with generalisations to include secondary extinction as noted above) clearly shows that, in regard to the reflected intensity, measurement in the symmetrical position, $\alpha = 0^\circ$ —the standard procedure with extended-face crystals since 1914 (ref. 12)—involves the maximum interaction associated with multiple diffraction and therefore the maximum extinction.

As a practical illustration of the procedure discussed here, the results for a 'strong reflection', the 200 of LiF with CuK α radiation, are presented. For this reflection, $\theta \approx 22.5^\circ$. A single-crystal boule was sawn at $\sim 19.5^\circ$ to the (200) plane, the surface ground and then given a finish flat to approximately one fringe in the working area. Measurements of intensity were made over the range $\alpha = -19.5^\circ$ to $+19.5^\circ$ (see ref. 8). After these were completed, a series of etches with HF were carried out, with intensities at selected tilt angles being measured after each etch. The intensity magnitudes dropped steadily and etching was continued until the values effectively stabilised. The state of the crystal surface was thus confirmed to be associated with secondary extinction. By contrast, the minimum value of intensity (measured at $\alpha = 0^\circ$), attained after etching was completed and thus relevant to the interior of the crystal, corresponded to high primary extinction, that is, the crystal as supplied was near 'perfect'. In support of this contention, the base value for the matrix is shown as the solid circle in Fig. 3; the value lies close to the theoretical value at the symmetrical position of curve (1) which was calculated for $F(200)$ of LiF and CuK α radiation for a 'perfect' crystal with the attenuation coefficient, $\mu = 32.2 \text{ cm}^{-1}$, using the formulae in Hirsch and Ramachandran⁵. Line (2) corresponds to the theoretical mosaic crystal with zero secondary extinction. Curve (3) corresponds to the experimental measurements on the optically-flat asymmetric LiF crystal. A simple function was found to fit the 10 experimental points from $\beta = 0$ to $|\beta| = 0.845$, and this function was extrapolated to $|\beta| = 1$. One may note that the simple mean of $\rho(+\beta)$ and $\rho(-\beta)$ can be used.

While the above results establish the basic principle of this method, it will be clear that there is considerable scope for the exploration of both its experimental and theoretical aspects, especially close to the limits $\alpha = \pm\theta$ where the refractive index may require careful consideration, and a full report will be presented elsewhere.

Fig. 2 Transformation of Fig. 2 of Hirsch and Ramachandran⁴ to show the functional dependence on the asymmetry factor β (defined as $\cot \theta \tan \alpha$) of the normalised integrated intensity ratio, $\rho(\beta)(1-\beta)^{-1}/\rho_0'(0)$, and of the normalised specific intensity ratio, $J(\beta)(1+\beta)^{-1}/J_0'(0)$. The line (a) is for the ideal mosaic crystal (with zero secondary extinction). Lines (b), (c), (d) and (e) are for a perfect crystal with $\lg(0)$ values as designated in Fig. 2 of ref. 4. Line (f) corresponds to the 200 reflection of LiF using CuK α radiation, and has been calculated on the basis of the formulae in ref. 4. The high primary extinction of this reflection for these conditions is evident.



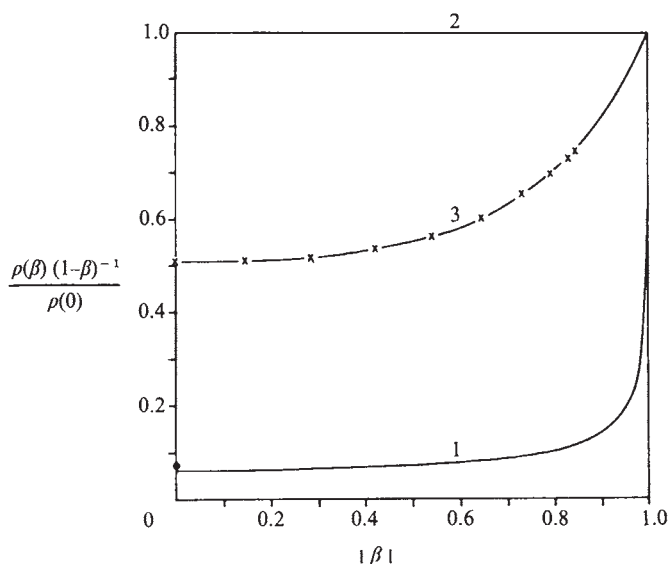


Fig. 3 Comparison of numerical data, experimental and theoretical, for the 200 reflection of LiF, scaled against the kinematical value as 1.0. The value, measured at the symmetrical position $\beta = 0$, for the single crystal after serial etching to reach the minimum intensity corresponding to the unstrained specimen, is indicated by the solid circle. Curve (1) is calculated for a perfect crystal of LiF, and corresponds to curve (f) in Fig. 2. Line (2) corresponds to the theoretical mosaic crystal with zero secondary extinction and is identical with line (a) in Fig. 2. Curve (3) shows the experimental points and their extrapolation to 1.0 at $\beta = 1$.

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Petroleum hydrocarbons inhibit decomposition of organic matter in seawater

MOTILE marine bacteria, like higher animals, have the capacity to detect and be attracted to organic substances¹, including living microbial prey². Mitchell *et al.*³ found that this chemotactic response was inhibited by low concentrations of hydrocarbons such as toluene, phenol and crude oil. The bacterial population was not killed but did lose its ability to detect non-living substrates as well as living prey, such as algae and enteric bacteria^{3–5}. It seems that the ability of a marine bacterium to degrade a substrate is augmented by its attraction to that substance. This report describes the role of bacterial chemotaxis in organic matter

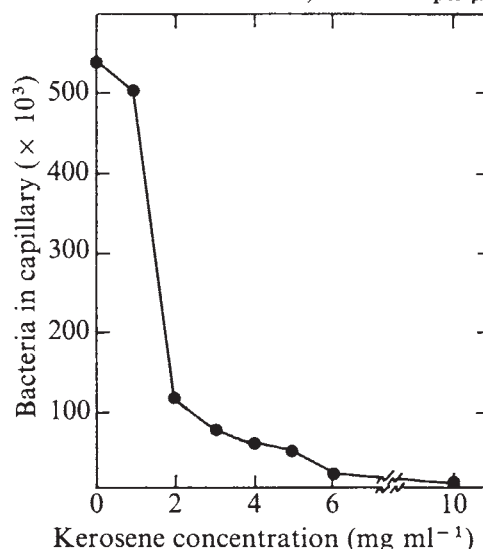
decomposition in seawater and its inhibition by sublethal concentrations of petroleum hydrocarbons.

Marine motile bacteria, which are capable both of utilizing either albumin or casein as a sole carbon and nitrogen source and being attracted to them, were isolated from seawater using an enrichment technique⁶. Their chemotactic response towards soluble albumin or casein was tested using the method developed by Adler⁷. To test the ability of the bacteria to detect particles of organic matter in the seawater, the two test substrates were denatured by boiling at 100 °C for 10 min. The denatured products were only slightly soluble. Samples of 0.3 g albumin or casein were placed in nylon bags with 0.1-mm pores, permitting completely free contact of the bacteria in the water with the substrates. Bacterial suspensions (5 ml; 10^8 cells ml⁻¹) were placed at a distance of 12 cm from the substrate in natural seawater. The bacteria were attracted to the albumin or casein and utilised them as carbon and nitrogen sources. Enzymatic decomposition of the organic matter was determined both gravimetrically and by chemical analysis of protein using the method of Lowry *et al.*⁸. The role of chemotaxis in this process was estimated by blocking the chemoreceptor with the addition of petroleum hydrocarbons to the water. Kerosene was used as the source of petroleum hydrocarbons. Emulsification was achieved by sonification of the kerosene in the water before the addition of the bacteria and the organic matter. Figure 1 shows the effect of kerosene concentration on the number of bacteria attracted to the capillary containing an attractant. The results reveal that a concentration above 1.2 mg ml⁻¹ kerosene significantly blocked the chemotactic activity of the bacteria. Motility was measured by monitoring the bacteria in a Petroff–Hauser counter under phase-contrast microscopy using a television camera and monitor to expand the scale. No inhibition of motility was observed within a range of kerosene concentration of 1.2–5.0 mg ml⁻¹. Above that concentration cell motility was adversely affected.

Figure 2 shows the effect of contact time on the blockage of chemoreceptors by kerosene. In this phase of the study we added 3.0 mg ml⁻¹ kerosene to the seawater. The data indicate that within 10 min almost maximal blockage is achieved. Neither decrease in motility nor increase in chemoreceptor blockage was observed after this time.

Table 1 reveals a significant inhibition of decomposition of both casein and albumin in seawater in the presence of 3 mg ml⁻¹ kerosene. This inhibition was overcome when the water was fully mixed using a magnetic stirrer. These

Fig. 1 Effect of kerosene concentration in seawater on the chemotactic response of marine motile bacteria towards albumin. Attraction to seawater alone: 6,500 bacteria per μ l.



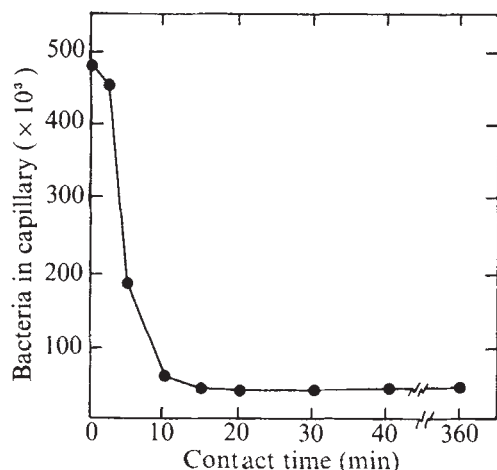


Fig. 2 Effect of contact time of marine bacteria with 3 mg ml⁻¹ kerosene in seawater on the blockage of bacterial chemoreceptors. Attraction to seawater alone: 6,500 bacteria per µl.

data show that, when chemotaxis was blocked, and the water was not mixed, no significant degradation of either albumin or casein could be detected. When random contact of the bacteria with the substrate was caused by physical mixing, however, 39% of the albumin and 65% of the casein were enzymatically degraded in 48 h, compared with almost 6% for albumin and 7% for casein in the standing cultures containing kerosene.

Table 1 Percentage of organic matter decomposition in the presence of 3 mg ml⁻¹ kerosene after 48 h

Treatment	Albumin degradation (%)	Casein degradation (%)
Standing		
Sterile seawater	5.2	4.3
Seawater + bacteria	22.0	50.2
Seawater + bacteria + kerosene	5.8	7.4
Fully mixed		
Sterile seawater	8.8	10.0
Seawater + bacteria	30.5	48.4
Seawater + bacteria + kerosene	39.2	65.5

Our data show that: chemotactic detection is important in bacterial degradation of these substrates; and that the kerosene concentration used in our tests blocks this function without inhibiting bacterial enzymatic activity. The relatively higher degradation rate in the physically mixed cultures containing kerosene can be partially explained by aeration and material loss resulting from the mechanical stirring (Table 1). The bacterial population increased by one order of magnitude from 10⁶ ml⁻¹ to 10⁷ ml⁻¹ in all of our tests. The phenomena which we observed do not seem to be the result of differential bacterial growth rates.

It seems that in seawater with a relatively low concentration of organic matter, bacterial chemotaxis may have a significant role in nutrient recycling. In polluted water chemotaxis inhibition may become a long term ecological problem since it can seriously undermine the capability of the marine microflora to decompose organic matter.

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Vulnerability of spatial frequency channels in cerebral lesions

QUANTITATIVE studies of vision in patients with cerebral lesions pose a challenge to purely topographical concepts of cerebral organisation. Psychophysical measurements of the detectability of sinusoidal gratings (contrast sensitivity functions)¹ established that in the majority of patients detection of high spatial frequencies suffers most. The spatial frequency of a grating is the number of its alternating bars subtended in 1° of visual angle; the higher the number, the finer the pattern. There seems to be no obvious correlation between this loss of fine pattern discrimination and the aetiology or the exact localisation of a cerebral lesion. The neural basis of this loss is unknown in vision as with the other senses. Is it the result of specific vulnerability of those neurones of the primary visual pathways which are optimally responding to high spatial frequencies or to some defect of high order cognitive functions? In this report I present data on three patients who recovered from cerebral blindness. Serial evoked potentials and psychophysical detection of gratings of high frequency showed concurrent changes throughout recovery in one patient in whom they could be measured. This suggests that structures of the central nervous system which determine evoked potentials and psychophysical responses to high frequency gratings are either identical or similarly vulnerable. These data also suggest why mid- and high-spatial frequency channels of the primary visual pathways may be especially vulnerable to cerebral lesions.

There is abundant evidence that gratings with sinusoidal luminance distribution may be optimal stimuli for cortical neurones of the mammalian¹⁻⁴ (including the human⁵) visual system. These stimuli approximate or match the spatial substructure of the receptive field of neurones of the primary cortex⁶ and it has been suggested that neurones of similar spatial frequency optima constitute parallel "channels" of the visual system⁷. In the normal human psychophysical detection of gratings can be correlated with evoked potential measurements⁸⁻¹⁰. Studies of patients with cerebral lesions provide unique opportunities for investigating the effects of lesions on this correlation. In addition, evoked potential (EP) studies of patients can establish whether or not loss of fine grating detection occurs without damage to the primary visual pathways. If evoked potentials are intact whereas subjective detection suffers, then damage to higher order functions could be one possible explanation. If, however, evoked potentials and psychophysical functions change correspondingly, then it is not necessary to postulate damage beyond lesion of the primary visual pathways up to and including the primary visual cortex.

Over the years, 24 patients have been studied. In many of them evoked potentials and psychophysical measurements of grating detection are similarly affected by cerebral lesions¹¹. In most patients clinical changes do not allow extensive psychophysical measurements, but in one rare case, serial evoked potential and psychophysical measurements were possible during recovery.

A 64-year-old woman, awoke one night with headache and inability to see, and was taken to hospital. Her neurological examination was normal except that she made errors

in dates, although she was orientated to place. Her vision was almost limited to detection of light. Occasionally she detected large objects and gross motion. Pupillary responses were intact. Optokinetic nystagmus was present although irregular and asymmetrical when a regular inch tape stimulus was used and it was absent to a rotating drum stimulus. EEG, including alpha rhythm and brain scan were normal. Subsequent studies revealed that she suffered from cardiac arrhythmia with intermittent heart block and hypotension which most likely accounted for the sudden onset of cerebral blindness. Visual evoked potential measurements were obtained the morning after admission (Fig. 1). She was able to detect a 0.8 cycle degree⁻¹ grating pattern when she was directed to look straight at the screen in front of her. This stimulus of 70% contrast produced evoked potentials in normal configuration. Evoked potentials were indistinguishable from noise when she detected no patterns above 2.1 cycles degree⁻¹. During her stay in hospital she progressively recovered peripheral vision and at the same time her spatial contrast sensitivity to foveally presented gratings improved as well. The recovery was apparent in the evoked potential measurements (Fig. 1b) and psychophysical and evoked potential measures of spatial contrast sensitivity were in agreement (Fig. 2). The noteworthy feature of these measurements was that evoked potentials provided evidence of damage of the visual pathways by confirming her subjective deficits. EPs were present in normal configurations when the patient said she saw the pattern and they were absent when she claimed no pattern detection. Furthermore, when evoked potential was present

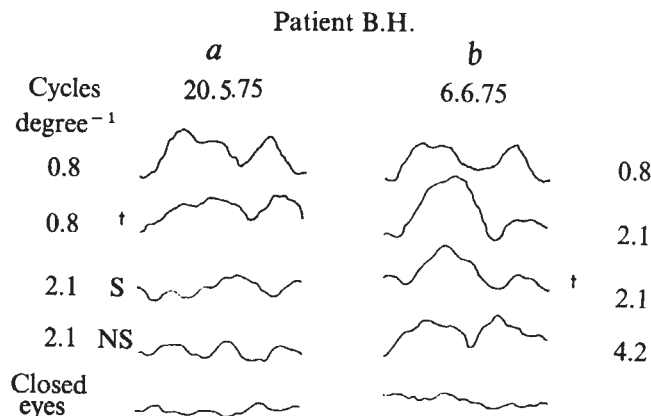


Fig. 1 Evoked potentials were recorded by silver disk electrodes placed on the scalp 2.5 cm above the inion and over the temporal bone corresponding to the posterior temporal electrode of the international 10-20 classification. The middle of the forehead was grounded. Potentials were filtered with corner frequencies of 1.5 and 60 Hz and averaged with a Nicolet 1062 computer, the active occipital electrode producing an upward deflection. Vertical gratings were generated according to standard methods^{12,11} on the CRT of an oscilloscope screen at the mean luminance of 40 foot lamberts. The diameter of the screen subtended 4° at the eye. The stimulus grating was shifted to and fro at the rate of 8 Hz by a distance corresponding to the width of a single bar of the pattern called "counterphase flicker"¹³. *a*, One day after the onset of cerebral blindness the patient detected a counterphase flickering grating of 0.8 cycle degree⁻¹ of 70% contrast as well as 20% contrast († symbol). She occasionally detected a 2.1 cycles degree⁻¹ grating of 70% contrast (S) to the left of the trace when she detected it; NS when she could not detect it. She could not detect any finer patterns that day. The duration of each trace is 125 ms and represents the average of 512 responses, or a recording time of 64 seconds. *b*, Marked improvement 17 d later. Evoked potentials were present to finer patterns than before, up to 10 cycles degree⁻¹ (not shown). †, EP to a 2.1 cycles degree⁻¹ counterphase grating of 20% contrast. Other traces obtained at 70% contrast. The number at the right is the spatial frequency of the pattern. The last trace was obtained when the patient closed her eyes. Calibration the same as (a).

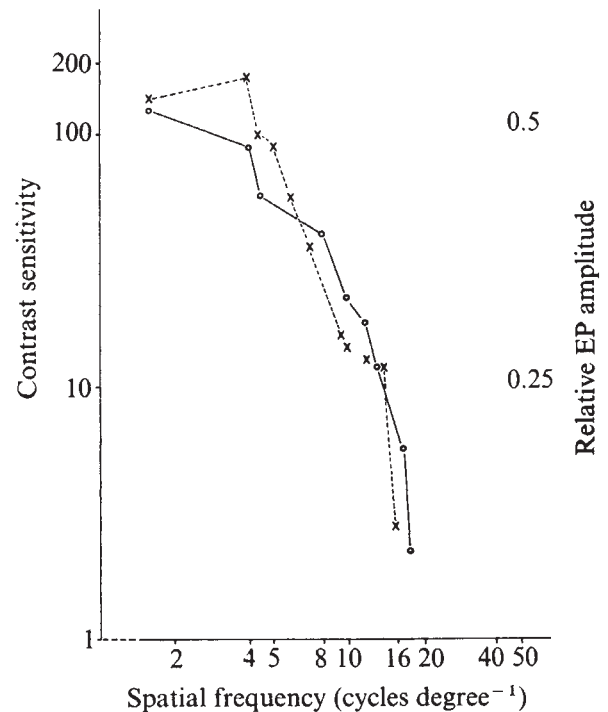


Fig. 2 Psychophysical and evoked potential measurements taken concurrently 5 weeks after admission. Psychophysical contrast sensitivity (O) and EP amplitude (X) are plotted as a function of spatial frequency. As our standard procedure¹⁰, the method of constant stimuli was used to obtain frequency of detection curves. The psychophysical threshold is taken as the 50% detectable contrast. The inverse of contrast threshold is called contrast sensitivity. EP amplitude was measured as the peak-to-trough average of the quasi-sinusoidal waveform which is constant for 8 Hz counterphase stimulation, as shown in Fig. 1 for previously obtained data. Five weeks after admission there is still a considerable loss in contrast sensitivity compared with normal. This loss, expressed as a ratio of the normal to the patient's contrast sensitivity (not shown), increases with spatial frequency.

there was correlation (as in normals⁸) between EP amplitude and the contrast of the pattern.

On admission to hospital the examination revealed that although the patient's peripheral vision and foveal fine pattern detection were absent some detection of crude patterns by foveal vision and occasional motion detection by peripheral vision remained. This suggests that there was damage to wide cortical areas, including peripheral "representation", but some foveal functions were intact. Potentials evoked by grating patterns recorded with the present method are largely determined by foveal contribution⁸; thus their presence soon after admission also suggests that some part of foveal "representation" was spared. The preservation of low but loss of high spatial frequency detection in the present case could be explained by the topography of lesions if detection of low and high frequency gratings by foveal vision were located in different areas of the calcarine cortex. Another possible explanation could be that representation of low spatial frequencies in parastriate regions received direct geniculate input (bypassing the primary cortex). Anatomical studies of the functional organisation of the visual cortex in primates do not provide support for these two possibilities¹⁴⁻¹⁶. Post-mortem studies in humans¹⁷ show that grey more than white matter, the occipital pole more than the anterior part of the calcarine cortex, show damage after cardiac arrest, or after a short period of lowered blood pressure. Histological examination reveals laminar necrosis of layer IV (ref. 18).

As an alternative to topographical concepts another possibility must be considered. Single-cell studies^{2-4,19} provide evidence that separate neurones with overlapping receptive

fields respond to different grating patterns. This so-called spatial frequency selectivity characterises not only primate, but also human cortical organisation¹. In this, as in other cases¹, recovery from cerebral lesions occurs *pari passu* with recovery of crude to finer pattern detection. This could occur if neurones optimally responding to low spatial frequencies were more numerous or had the greatest resistance to hypoxia or both. There is evidence that neurones of low spatial frequency optima belong to a functionally¹⁰ different class than those with high spatial frequency optima but there is no evidence that there is a difference in numbers⁹. In addition to differences in function these two classes of neurones seem to have different neuropharmacological requirements^{10,21}.

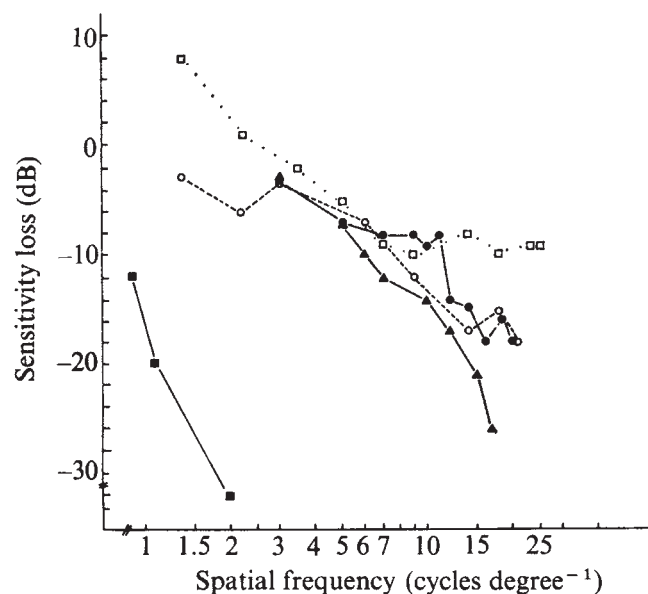


Fig. 3 Five visuograms demonstrate high frequency losses in three patients who recovered from cerebral blindness. A visuogram is the plot of the loss of the detectability of gratings, expressed in decibels as the ratio of the normal to a patient's contrast threshold at corresponding spatial frequencies. The gratings were presented in an on/off manner at the rate of 0.5 Hz. The degree of recovery of fine grating detection appears to be correlated with the time elapsed since the onset of blindness for an individual patient (dark symbols) and between patients. Solid symbols represent the data of the patient reported in detail on admission (■), two (▲) and four (●) weeks later. Empty circles show data of a 71-yr-old woman, and empty squares those of a 23-yr-old woman, respectively, 5 months and 5 yr after the onset of cerebral blindness.

This case and the two others summarised in Fig. 3 suggest that damage to neurones of the primary pathways which may be especially vulnerable to hypoxia, could explain high spatial frequency losses in humans. It remains to be seen whether or not metabolically vulnerable neurones of high spatial frequency optima can be identified in primates.

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Simulation *in vitro* of bovine host cycle of *Theileria parva*

Theileria parva is a tick-transmitted parasite causing East Coast fever in cattle. Research on *T. parva* has been impeded because of difficulties in completing the mammalian part of its life cycle in any non-bovine host. Several attempts to adapt the organism to small laboratory animals have failed¹⁻⁵. Limited success was reported by Irvin *et al.*^{6,7} using whole-body irradiated nude athymic mice. Many attempts to cultivate the parasite *in vitro* also failed⁸⁻¹² until Malmquist *et al.*¹³ were successful using suspension cultures of infected bovine lymphoid cells which eventually contained the macroschizont form in the cytoplasm—the relationship between bovine lymphoid cell and parasite makes the perpetuation of both possible in a suitable culture medium. This led to further research into the development of an artificial immunogen and studies of the macroschizont stage of the life cycle without recourse to the bovine host. So far, however, tissue culture methods have not produced all stages of the parasite subsequent to the macroschizont; namely microschorizonts, micromerozoites and piroplasms. The final stage, which is intraerythrocytic, is believed to be of crucial importance in initiating the separate cycle which occurs in the tick vector (*Rhipicephalus appendiculatus*).

An infected bovine lymphoid cell line, established by the method of Malmquist *et al.*¹³ from a prescapular lymph node biopsy, was used in the work reported here. Cultures were maintained in Eagle's MEM with Earle's salts, supplemented with 10% foetal calf serum (FCS) plus 0.1% extra L-asparagine. Experimental variations of this standard culture medium showed that, by adding bovine lymph 5-10% and increasing the FCS to 20 or 30%, cells and parasites were stimulated to produce microschorizonts, micromerozoites and, when bovine red cells were added to the cultures, piroplasms. The presence of the bovine lymph seemed to be associated with acceleration of mitosis of host cells with production of the later stages of the parasite as found in the bovine host. Mitosis of the cell was seen only when the parasite was in the macroschizont stage.

In one experiment it was shown that 50, 20 or 10% lymph added to cultures without FCS was toxic to the cells and an initial inoculum of 5×10^5 viable infected cells was completely non-viable by day 5. The addition of 20% FCS to medium containing 20 and 10% lymph seemed to provide the cells with sufficient nutrient to cope with the accelerated mitosis and parasite division with acceptable viable cell counts on day 5. The production of stages of the parasite other than macroschizonts was observed in smears stained with Giemsa. The utilisation of the nutrient material in the culture medium was emphasised in a further experiment. On day 3 half of each culture was withdrawn and added to fresh medium in a new culture flask. The viable cell counts of the original cultures continued to decline, whereas those provided with fresh medium showed at least a twofold increase after a further 2 d. Microschi-

zonts have been observed at day 2 in such cultures and micromerozoites at day 3, except for one instance when they appeared at day 2. Ten experiments were set up in which washed bovine red cells were added to cultures of macroschizont-infected cells in medium containing bovine lymph. Microschizonts, micromerozoites and piroplasms were observed in 9 of these, of which 7 were 18-h cultures at the time of addition of the red cells, and the other two were 42- and 66-h cultures. The tenth culture was 90 h old before the red cells were added and there was no development of the parasite beyond the microschizont stage.

In smears made from the 9 cultures in which piroplasms were observed, only a small number of these were seen in each smear; estimated at 0.1% of red cells. Piroplasms were seen in these cultures in smears made over a period from 6 to 54 h after addition of red cells.

The intraerythrocytic location of the piroplasms was determined on the basis of shape and position of the parasites characteristic of those seen in the red cells of *T. parva*-infected cattle. In a few preparations an occasional micromerozoite was seen in an indentation of the envelope of a red cell, apparently in the process of entering the cell. Neither intraerythrocytic parasites nor parasites apparently in the process of entering the red cell were seen in any of the control cultures containing red cells and no lymph.

The above work shows that development of the parasite to its ultimate intraerythrocytic stage is possible *in vitro*. Experiments with further changes in the culture medium and/or conditions of culture may lead to a higher proportion of infected red cells in tissue culture, making the transfer of such infected material to the tick vector easier both *in vivo* and *in vitro* so that the whole life cycle of *T. parva* may be completed outside the bovine host.

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Cell-mediated cytotoxicity to SV40-specific tumour-associated antigens

CYTOTOXIC thymus-derived lymphocytes (T cells) from mice infected with lymphocytic choriomeningitis virus, ectromelia virus, vaccinia virus, or parainfluenza virus interact only with H-2 compatible virus-infected target cells¹⁻⁶. When congenic H-2 recombinant mice⁷ and mice containing mutations within genes mapped at H-2K end specificities^{8,9} are used, recognition of virus-infected target cells by the effector lymphocytes requires compatibility at either the K or D locus of the H-2 gene complex. These observations led to the hypothesis that cytotoxic T cells recognise either a complex of viral and histocompatibility antigens

or virus-induced alterations of the histocompatibility antigens. The same restriction has been described for the cytotoxic T cell response to lymphocytes modified by trinitrophenyl (TNP)⁹, to minor histocompatibility antigens^{10,11} and to the male Y antigen¹². To investigate the possibility of a similarly restricted specificity of the effector cells of cell-mediated immunity to tumours, cytotoxic cells specific for SV40 tumour-associated specific antigens (SV40-TASA) were generated in two inbred strains of mice, C57BL/6J and BALB/cAn. The SV40-transformed lines C57SV (SV40-transformed C57BL/6 mouse embryo fibroblasts), MKS/Bu100 (SV40-transformed BALB/c kidney cells¹³) and LN-SV (SV40-transformed human skin fibroblasts¹⁴) were used as immunising and target cells (Table 1). In

Fig. 1 Time course of the generation of effector cells in mice immunised with SV40-transformed cells. C57BL/6J mice were immunised with a single intraperitoneal injection of 3×10^7 syngeneic SV40-transformed mouse-human somatic cell hybrid C121 (mouse parental C57BL/6 macrophages, a), allogeneic SV40-transformed hybrid C136 (mouse parental BALB/c macrophages, b) or xenogeneic SV40-transformed human cell LN-SV (c). The cytotoxic activity of spleen cells was tested on different days after immunisation. The target cells were: syngeneic C57SV (SV40-transformed C57BL/6 mouse embryo fibroblasts, ○), allogeneic MKS/Bu100 (SV40-transformed BALB/c mouse kidney cells, ●), and xenogeneic LN-SV (SV40-transformed human fibroblasts, □). Dose-response curves of specific cytotoxicity were determined by plotting ⁵¹Cr-specific release compared with number of lymphocytes expressed in log₁₀ units. The number of lymphocytes necessary to lyse 50% of 2×10^4 target cells in 12 h of incubation is referred to as 1 lytic unit.

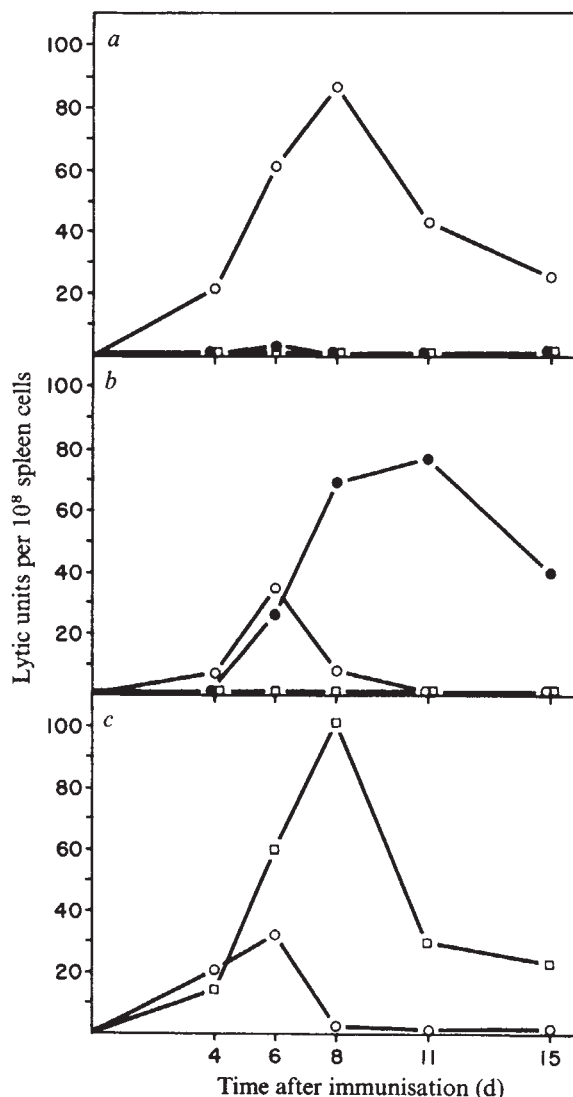


Table 1 *In vitro* cell-mediated cytotoxicity by spleen cells of mice immunised with syngeneic and xenogeneic SV40-transformed cell lines

Target cells	SV40 T antigen	Strain of origin	Human chromosomes	Responder strain	C57BL/6J	C57BL/6J	C57BL/6J	BALB/cAn
				Immunising cells	C57SV	C121	LN-SV	C136
				Time after immunisation (d)	8	8	6	8
				Incubation time (h)	12	12	18	20
C57SV	+	C57BL/6J	—		54	68	61	0
MC57G	—	C57BL/6J	—		0	3	4	0
C57BL EF	—	C57BL/6J	—		NT	NT	3	NT
C121	+	C57BL/6J	7		33	49	54	5
N9*	+	C57BL/6J	7		28	NT	57	NT
N8*	+	C57BL/6J	7		49	NT	60	NT
243B†	—	C57BL/6J	+		6	NT	NT	NT
MKS/Bu100	+	BALB/c	—		6	4	39	21.1
BALB EF	—	BALB/c	—		5	6	8	0
BALB/3T3	—	BALB/c	—		0	NT	5	2
C136	+	BALB/c	7		2	0	NT	37.8
NIH-SV3T3	+	Swiss random bred	—		NT	NT	25	NT
NIH-3T3	—	Swiss random bred	—		NT	NT	3	NT
LN-SV	+	All	All		8	0	59	2

C57BL/6J and BALB/cAn mice, 8–12 weeks old, were given a single intraperitoneal injection of 3×10^7 trypsinised cells. The effector cells were obtained from spleens of groups of at least three animals by gentle dissociation in a loose Potter homogeniser. The cytotoxic tests were performed in triplicate in the wells of flat-bottomed microtitre plates. Target cells were trypsinised and 2×10^4 cells and $2 \mu\text{Ci } ^{51}\text{Cr}$ were added to each well. After 18 h the target cells were washed and effector cells in twofold falling dilutions were added in 200 λ medium. After suitable incubation times half the supernatant was removed for counting; the target cells were lysed with 1% Triton X-100 to measure the total releasable ^{51}Cr in each well. ^{51}Cr specific release, at a 100:1 ratio of effector to target cells was calculated according to the formula:

$$\%^{51}\text{Cr specific release} = \frac{\left(\frac{\%^{51}\text{Cr release in the presence of immune cells minus } \%^{51}\text{Cr release in the presence of non-immune cells}}{100 \text{ minus } \%^{51}\text{Cr release in the presence of non-immune cells}} \right) \times 100}$$

The standard error was usually less than 1%. The ^{51}Cr release in the presence of non-immune cells was always either very close or identical to the release in medium alone, and ranged in the different target cells from 1.5 to 2.5% per h.

NT, Not tested.

* Clones of somatic cell hybrids obtained by fusion of LN-SV with C57BL or BALB/c peritoneal macrophages.

† Somatic cell hybrid obtained by fusion of an adeno-5 virus transformed human line²² and C57BL/6 peritoneal macrophages.

addition, clones of human-mouse somatic cell hybrids, obtained by fusion of LN-SV and mouse peritoneal macrophages from inbred strains¹⁵ were used. These hybrid clones contain the complete mouse genome and, in addition, one to several copies of human chromosome 7, in which the SV40 genome is presumably integrated^{14,15}. C121, N8 and N9 cells are derived from LN-SV fused with C57BL/6 macrophages¹⁶ and C136 cells from LN-SV fused with BALB/c macrophages¹⁷. These clones express the SV40 tumour (T) antigen (a nuclear antigen)^{16,17}, and the histocompatibility antigens of the murine parental cells (our unpublished results). Neither LN-SV nor any hybrid clones derived from LN-SV make infectious SV40¹⁸. Only after fusion with permissive monkey kidney cells can some SV40 particles be rescued. This defective virus exhibits no biological activity. Both LN-SV and human-mouse somatic cell hybrids containing the human chromosome 7 derived from LN-SV exhibit tumour-specific transplantation antigens (TSTA), as demonstrated by their ability to protect SV40-inoculated newborn hamsters from developing SV40 tumours¹⁹. Both the SV40-transformed mouse cell lines (our unpublished results) and the hybrid clones^{16,17} are tumorigenic in immunodeficient "nude" mice, but these cells do not grow routinely in immunocompetent syngeneic mice, probably because of strong TSTA²⁰.

Mice were given a single intraperitoneal injection of 3×10^7 syngeneic transformed mouse cells or syngeneic mouse-human somatic cell hybrids. This led to the generation of effector cells in the spleen, with a peak of cytotoxic activity, as measured by ^{51}Cr release assay, 8 d after immunisation (Fig. 1a and Table 1). The cytotoxic cells were specific for syngeneic SV40-transformed cells. The cytotoxic activity was removed by treatment of the spleen cell preparations with anti-Thy 1,2 serum (Searle; AKR anti-C3H thymocyte sera) and complement. Allogeneic and xenogeneic cells, either transformed or not, were not affected by exposure to effector cells (Table 1). Cells from a syngeneic methylcholanthrene-induced tumour (MC57G),

spontaneously transformed embryo fibroblasts (C57BL EF and BALB EF) and a hybrid clone from fusion of C57BL/6 macrophages with human adeno-5 virus-transformed human cells (expressing adeno-5 T antigen, F. K. Huebner, personal communication) were also not lysed (Table 1). The cytotoxic response generated in C57BL/6 mice was always several times higher than that observed in BALB/c mice; when BALB/c effector cells were used, a significant level of cytotoxicity, with restriction for SV40-transformed syngeneic cells, was observed only at longer incubation times (15–20 h, Table 1, column 4).

When the mouse-human somatic cell hybrids are used as immunogens they induce effector cells specific for SV40-transformed syngeneic mouse cells. When these hybrid cells are used as targets they are killed by cytotoxic cells induced by immunisation with syngeneic SV40-transformed mouse cells. Therefore these cells, which carry the SV40 genome integrated in the human chromosome 7, fully express SV40-TASA, which can interact with the mouse alloantigens to allow recognition by the effector cells. Additionally, immunisation of mice with these mouse-human hybrid cells does not result in generation of effector cells directed to surface antigens coded by human chromosome 7 since there is no cell-mediated cytotoxicity of human cells. These negative results contrast sharply with those obtained using sera of H-2-compatible mice hyper-immunised with the same human-mouse hybrid cells where significant antiserum activity to human cells is found²¹. The possibility remains that cytotoxic effector cells are generated specifically for the interaction between the human chromosome 7-coded antigens and the mouse alloantigens, but this cannot be demonstrated because of the simultaneous strong response to SV40-TASA on the hybrid target cells used.

The time course and peak of cytotoxic activity in the spleen of an animal immunised with SV40-transformed syngeneic cells (Fig. 1a) corresponds to that observed with xenogeneic antigens (Fig. 1c), and is slightly earlier than

the peak of activity to alloantigens (Fig. 1b). After immunisation with SV40-transformed allogeneic (Fig. 1b) and xenogeneic cells (Fig. 1c), any specificity for SV40-TASA on allogeneic or xenogeneic cells is masked by the simultaneous peak of cytotoxic activity against alloantigens and xenoantigens. The murine response to SV40-TASA, however, can be dissected in the allogeneic immunisation by the use of SV40-transformed syngeneic cells; that is when syngeneic mouse target cells are used, a peak of activity is seen 6 d after immunisation (Fig. 1b). After immunisation with SV40-transformed human cells, this cytotoxic response at day 6 is specific for SV40-transformed mouse cells (or mouse-human hybrids), regardless of their alloantigens (Fig. 1c, Table 1, column 3). This early cytotoxic response to syngeneic transformed cells after xenogeneic immunisation appears to be a T-cell response because it is (1) abrogated after complement-dependent lysis of effector cells with anti-Thy 1.2 sera, and (2) enhanced on a per cell basis after the effector cell population is eluted from a nylon wool column to remove adherent cells and significantly reduce the number of immunoglobulin-bearing cells.

The population of effector cells specific for SV40-TASA generated during immunisation with SV40-transformed syngeneic cells is apparently different from that generated by immunising with SV40-transformed allogeneic or xenogeneic cells. This hypothesis is based on two observations: (1) there is a different time course of cytotoxic activity against SV40-transformed cells after immunisation with allogeneic and xenogeneic cells (as compared with that after immunisation with syngeneic cells); and (2) there is a different specificity of the effector cells (which in the case of xenogeneic immunisation is not restricted to syngeneic target cells). LN-SV and its somatic cell hybrids cannot produce infectious SV40 in the mouse as the transforming virus is defective and mouse cells are not permissive for SV40 replication. Therefore, the immune response observed using allogeneic and syngeneic cells as targets is not due to the infection of host cells with subsequent expression of SV40-TASA. Moreover, any active infection of the immunised animal would result in the production of effector cells which could recognise only syngeneic target cells.

Our results indicate that functional interaction between alloantigens and new antigens on cell surfaces is necessary for the generation of effector T cells not only to viral antigens expressed during acute infection, but also to TASA expressed by transformed cells. Moreover, in the SV40 system, the degree of the cytotoxic response against tumour-associated antigens obtained with syngeneic immunising cells and target cells is comparable with that observed against allogeneic histocompatibility antigens. This syngeneic cell-TASA response is at least three times greater than that obtained by immunisation with allogeneic or xenogeneic SV40-transformed cells, using syngeneic SV40-transformed cells as targets.

Cytotoxic effector T cells may have a role in immunosurveillance against tumours. *In vivo* H-2-dependent restriction of the specificity of cytotoxic effector cells might be involved in the immune control of tumour growth, enhancing the cytotoxic response of the host. This augmentation of the immune response to tumour-associated antigens might be heightened in normal outbred populations due to the heterozygosity for histocompatibility antigens, each of which can interact with the newly expressed antigens on tumour cells, eliciting different clones of cytotoxic effector cells²².

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Alteration in membrane glycoproteins after type-C virus infection of murine fibroblasts

New proteins and glycoproteins appear on cell membranes after infection of the cells by type-C viruses¹. Some of these new proteins are virus coded and are positioned in the membrane away from the sites of virus budding². GP70, the major murine glycoprotein has been identified serologically on the membranes of virus-shedding cells³, transformed but non-virus-producing cells⁴, and on some chemically induced sarcomas⁵. The internal core protein p30 has been identified as a common antigen on membranes of type-C virus-infected cells of many species⁶. It has been suggested that this protein, possibly a product of degraded virus, can bind nonspecifically to virus-shedding cells⁵. Non-virion virus-coded antigens have also been described. The best characterised tumour-specific surface antigen (TSSA) is that found on avian sarcoma cells⁷. It consists of a major fucose-containing glycoprotein of molecular weight 100,000 and possibly a minor glycoprotein of 32,000. These glycoproteins do not cross react antigenically with the avian virion glycoproteins. A similar antigen, the feline oncornavirus membrane antigen (FOCMA), has been described on virus-infected feline cells⁸. This antigen has not been well characterised but it is also thought not to be related to the virion proteins. Evidence has been sought largely unsuccessfully for a comparable TSSA in murine model systems⁹. There have, however, been a few reports of sarcoma specific antigens¹⁰. In this paper we describe alterations which occur in membrane glycoproteins after infection of murine fibroblasts by Moloney leukaemia-sarcoma virus (MSV-MLV-M) and by an N-tropic virus isolated from leukaemic AKR mice (MLV-AKR). The technique of lectin chromatography is used to fractionate glycoproteins from other membrane proteins enabling better characterisation of these important surface molecules.

Virus was isolated from tissue culture fluids by sucrose gradient centrifugation. ³H-leucine-labelled cells were disrupted by nitrogen cavitation and the plasma membranes prepared by the two-phase method of Brunette and Till¹¹.

The virus and membrane samples were then dissociated with 1% deoxycholate (DOC) in water and glycoproteins were isolated and partially fractionated by affinity chromatography using *Lens culinaris* (LcH) lectin which is specific for α -D-mannopyranosyl-like residues¹² and *Ricinus communis* (RCA₁) lectin, which is specific for β -D-galactopyranosyl-like residues¹³. The agglutinins were bound to cyanogen bromide activated Sepharose 4B (ref. 12).

Figure 1 shows the fractionation of MSV-MLV-M virus on LcH and RCA₁ affinity columns. The major viral glycoproteins of Moloney virus bind to both LcH and RCA₁ lectin columns and are resolved into two components on SDS gels having apparent molecular weights of 74,000 and 79,000 (Fig. 1i and j). Figure 1i, j, e-h shows that the selective binding of glycoproteins to the LcH and RCA₁ affinity columns separates the viral glycoproteins from proteins of similar electrophoretic mobility and apparent molecular weight which are presumably non-glycosylated. For example, proteins of approximately the same apparent molecular weight as the viral glycoproteins have been identified as precursors of the non-glycosylated viral group-specific antigens^{15,16}. The ability of lentil lectin to discriminate between glycoproteins and non-glycosylated proteins was verified by using the periodic acid-Schiff (PAS) technique²⁰ to detect carbohydrate containing proteins on a sodium dodecyl sulphate (SDS) gel of lentil lectin fractionated Moloney virus (data not shown). Therefore, this simple procedure facilitates the isolation of viral glycoprotein. A glycoprotein of the MLV-AKR virus was isolated in a similar manner and found to be a single entity of molecular weight 79,000, which bound to both LcH and RCA₁ lectin columns (data not shown).

Infection of secondary cultures of mouse embryo fibroblasts (Taylor's Own mice (TO), ICRF) with MSV-MLV-M results in the appearance of three new glycoprotein bands revealed by SDS gel analysis of the membrane preparations. Two of these glycoproteins bind equally well to RCA₁ and LcH columns and have molecular weights of 74,000 and

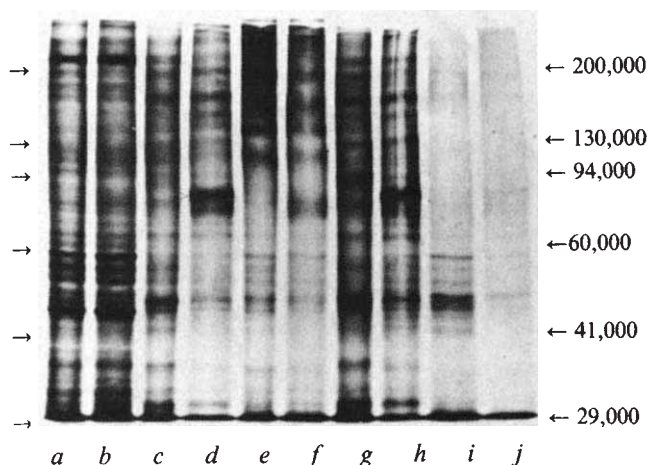


Fig. 2 Autoradiogram of 8% SDS-PAGE of membrane fractions of TO fibroblasts and TO fibroblasts infected with MSV-MLV-M (TO-M) after chromatography on columns of LcH and RCA₁ lectins. Cells were grown in Dulbecco's modified Eagle's medium with 10% foetal calf serum, and labelled for a 24-h period before collecting with ³H-leucine (5.0 mCi; in 50 ml medium on 10 × 90 mm Petri dishes). Cells were collected by scraping, mixed with an excess of unlabelled SV40 B3T3 cells, washed with phosphate-buffered saline (PBS) and plasma membrane prepared by the two-phase polymer procedure of Brunette and Till¹¹. The membranes were suspended in 1% DOC and the rest of the procedure was as in Fig. 1. 'Unbound' samples containing 150,000 c.p.m. and 'bound' samples containing 100,000 c.p.m. were applied to the SDS gel. After staining, the gels were impregnated with 2,5-diphenyloxazole (PPO)¹⁸, exposed at -70 °C to Kodirex RP/R54 X-ray film for 5 d. Samples: a, TO; b, TO-M plasma membrane preparations; c, TO; d, TO-M lentil-bound fractions; e, TO; f, TO-M ricin-bound fractions; g, TO; h, TO-M ricin-unbound → lentil-bound fractions; i, TO; j, TO-M lentil-unbound → ricin-bound fractions. Standards (not shown) used are as described in Fig. 1 except that horse alcohol dehydrogenase (molecular weight 41,000) has been used in place of ovalbumin.

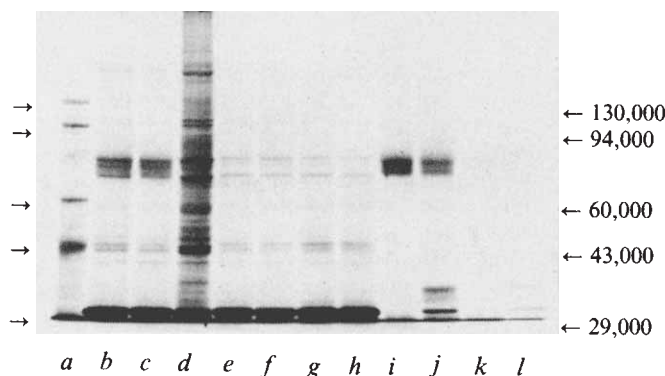


Fig. 1 8.0% SDS-polyacrylamide slab gel electropherogram (PAGE) of MSV-MLV-M fractionation on columns of LcH and RCA₁ lectin. MSV-MLV-M (5.0 mg) was disrupted in 12 ml 1% DOC (H₂O), sonicated for 30 s at 4 °C at position 4 on a Dawe Soniprobe (Type 7530 A) b, Centrifuged at 100,000g at 4 °C for 1 h (c, supernatant; d, pellet). A 5.0-ml sample of supernatant was applied to each of the lectin columns and washed through with 1% DOC (H₂O). The adsorbed glycoproteins were subsequently eluted with 0.3 M α -methyl-D-mannose (LcH) or 0.3 M D-galactose (RCA₁) in 1% DOC. The following samples were obtained: i, lentil bound; j, ricin bound; e, lentil unbound; f, ricin unbound; k, ricin unbound → lentil bound; l, lentil unbound → ricin bound; g, ricin unbound → lentil unbound; h, lentil unbound → ricin unbound. Bands at the bottom of tracks j and l represent eluted ricin. Standards (track a) used for the purpose of calculating molecular weights were: β galactosidase (130,000); phosphorylase a (94,000); catalase (60,000); ovalbumin (43,000); and carbonic anhydrase (29,000). S.d. of molecular weight calculations $\pm$ 1,300. The gel system used was that of Studier¹⁴. The gel was stained with Coomassie blue.

79,000 (Fig. 2d and f). The lectin-binding specificities and molecular weights of these two glycoproteins are identical to the glycoproteins isolated from the purified virions. The third new glycoprotein which appears in infected cell membranes has an apparent molecular weight of 84,500 and is bound only by lentil lectin (Fig. 2d and h). A similar result was obtained after infection of TO secondary fibroblasts with a second preparation of MSV-MLV-M isolated from tissue culture supernatants from the MSC cell line¹⁷.

Comparative analysis of the membrane glycoproteins of the N3T3 cell line before and after infection by MLV-AKR (Fig. 3) reveals changes in a triplet of lentil-binding glycoproteins, ranging in molecular weight from 80,000 to 88,000 (Fig. 2g and h). These proteins have similar molecular weights to the new lentil-binding protein described in membrane preparations of MSV-MLV-M (TO-M) cells (Fig. 2). On infection of N3T3 cells with MLV-AKR the concentration of this glycoprotein triplet increases threefold. This increase was quantified by densitometry measurements. There is no alteration in intensity in any of the other membrane glycoproteins. Moreover, this triplet of glycoproteins binds exclusively to lentil lectin, whereas the viral glycoprotein binds both to lentil and ricin lectin columns (Fig. 3f). A direct comparison of the effects of infection by MLV-AKR and MSV-MLV-M in TO cells was not possible as TO cells restrict replication of MLV-AKR.

The lentil lectin-binding glycoproteins are most probably related to infection by MLV rather than to transformation by MSV as the effect was observed in cells infected by MSV-MLV-M in which the number of transformants was less than 1% of the infected cells. Moreover, the non-transforming MLV-AKR caused an alteration in similar lentil-bound glycoproteins.

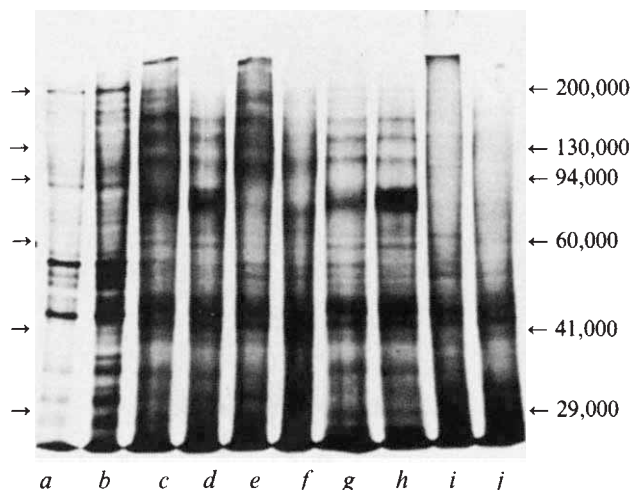


Fig. 3 Autoradiogram of 8% SDS-PAGE of membrane fractions of N3T3 cells and N3T3 cells infected with MLV-AKR after chromatography on columns of LcH and RCA₁ lectins. Samples were prepared as in Fig. 2. Samples: *a*, N3T3; *b*, N3T3-AKR plasma membrane preparations; *c*, N3T3; *d*, N3T3-AKR lentil-bound fractions; *e*, N3T3; *f*, N3T3-AKR ricin-bound fractions; *g*, N3T3; *h*, N3T3-AKR ricin-unbound → lentil-bound fractions; *i*, N3T3; *j*, N3T3-AKR lentil-unbound → ricin-bound samples.

This glycoprotein(s) may be a re-expressed embryonic antigen, a tumour-associated protein similar to the avian TSSA (ref. 7) or feline FOCMA (ref. 8) antigens, or it may be a precursor to the envelope glycoproteins similar to the precursor polypeptides which have been identified for the major non-glycosylated group-specific proteins of the virus^{18,19}.

Expression of endogenous virus and/or viral proteins in cell lines maintained in tissue culture is not an uncommon finding. It is possible that the expression of the lentil-bound 'triplet' of proteins seen in the N3T3 membrane sample in Fig. 3c is an example of such an event. After viral infection of the N3T3 cell line the concentration of this triplet of proteins seemed to increase threefold (Fig. 3g and h). This may be the result of infection by the endogenous AKR virus. It has also been suggested that infection by an exogenous oncogenic virus can stimulate derepression of endogenous virus²¹ and that the target specificity of the cellular immune response directed against the resulting tumour will be endogenous viral protein¹⁹.

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Genetic mimicry by hepatitis B virus

THE genetic hypothesis concerning the distribution of hepatitis B antigen (HB_sAg, Australia antigen) carriers was proposed by Blumberg¹ and states that susceptibility to the chronic asymptomatic carrier state is determined by a simple autosomal recessive gene (*Au*¹). Persons homozygous for this gene (*Au*¹/*Au*¹) should become chronic carriers of the virus when infected with hepatitis B virus (HBV), whereas those heterozygous (*Au*¹/*Au*⁰) or negative for the susceptibility gene (*Au*⁰/*Au*⁰) should not. Asymptomatic carriers of HB_sAg are presumably chronically infected with HBV. Although independent segregation analyses of HB_sAg in the families of blood donors^{2,3} and natural populations^{1,4-6} have failed to reject this hypothesis, it cannot account for the occurrence of a set of identical twins in which one twin had antigen, whereas the other had antibody²; nor for children with anti-HB_s or without antigen in families where both parents were carriers of HB_sAg (refs 3, 6 and 7). Indirect support for genetic control of the outcome of HBV infection comes from studies of thalassaemic patients in Italy⁸ and asymptomatic carriers in the Solomon Islands⁹. These have suggested that susceptibility to the chronic HB_sAg carrier state may be linked to heterozygosity of *Gm* genes and the resulting inability to synthesise as many kinds of anti-*Gm*. This hypothesis has not been supported, however, by subsequent investigations of other populations^{10,11}. On the basis of results presented here of segregation analysis carried out on data from the Solomon Islands, we have been able to reject the existence of a susceptibility gene as a major factor leading to the HB_sAg carrier state in this population.

We have carried out family and genetic studies of the distribution of HB_sAg and anti-HB_s in the total population of 771 individuals in Graciosa Bay on Santa Cruz Island in the Solomon Islands. The people were Melanesian, well nourished, and lived in leaf houses. They were apparently healthy at the time of sampling and were involved in normal village life. Reversed passive haemagglutination tests were carried out to detect HB_sAg using the Auscell system (Abbott) with microtitre equipment. Antibody was detected by a passive haemagglutination test using lyophilised HB_sAg-coated human red blood cells (Dr Lacy Overby, Abbott Laboratories). All positive reactions were confirmed by specific neutralisation by HB_sAg, or anti-HB_s, whichever was appropriate.

Seventy-seven families were studied in which both parents were tested. Antigen was found in the children of 19 families. These were subjected to segregation analysis using the 'maximum likelihood' method and the method of Li and Mantel¹². These analyses were carried out by Dr A. Barron, Department of Mathematics, Statistics and Computer Science of American University, Washington, DC.

Fifty-six children were investigated from 11 families in which both parents were negative for HB_sAg and at least one child was positive. The maximum likelihood method was used to estimate θ , the probability that any given offspring would be a carrier of HB_sAg (Table 1). We were testing the hypothesis that $\theta = 0.25$, which would be expected if the trait is an autosomal recessive. This analysis yielded a value of 0.182 ± 0.068 . The comparison of this distribution with that expected for an autosomal recessive gave $\chi^2 = 0.901$. This indicates no signi-

Table 1 Maximum likelihood analysis of the distribution of HB_sAg carriers on Santa Cruz

Parental type	No. of children	θ	Expected	Standard deviation	χ^2
Negative $\times$ negative	56	0.182	0.25	0.068	0.901
Negative $\times$ positive	26	0.244	0.50	0.103	5.959

ficant difference between the actual and expected distributions. The method of Li and Mantel gave an estimated θ of 0.167 ± 0.081 (Table 2). This is within 2σ of the expected value of 0.25 and therefore supports the conclusion of the maximum likelihood method. From these data it is not possible to reject the hypothesis that $\theta = 0.25$; therefore the trait of being an HB_sAg carrier is distributed as an autosomal recessive.

In the group in which one parent was negative and one was positive for HB_sAg and at least one child was positive there were 26 children in 5 families. The genetic hypothesis would predict a value for θ of 0.50. The maximum likelihood analysis gave a θ of 0.244 ± 0.103 and a χ^2 of 5.959 (Table 1). This is significant at the 2.5% level and indicates that the actual distribution in these families is significantly different from that expected of an autosomal recessive trait. This is in contrast to the results obtained with families of negative parents. The method of Li and Mantel gave an estimated θ of 0.182 ± 0.123 (Table 2). This is not within 2σ of the expected 0.50 and therefore supports the conclusions of the maximum likelihood method. On the basis of this analysis the hypothesis that $\theta = 0.50$ and that the trait is distributed as an autosomal recessive can be rejected.

There were three families in which both parents were antigen carriers. These people have remained carriers of the antigen for at least 2 yr. The genetic hypothesis would predict that all children of these matings who were infected must become carriers. Of the 13 children in this group, however, only two were carriers, three had antibody and eight were negative for both antigen and antibody. The three children with antibody have certainly experienced infections with HBV but did not become carriers. This is clearly not compatible with the genetic hypothesis. Additional evidence against the genetic hypothesis is offered by a family in which both parents were negative for HB_sAg and all their four children were carriers. The probability of this occurrence according to the simple genetic hypothesis is 1 in 256.

A basic assumption in segregation analysis is that the genotype is reflected in the phenotype. We must assume that all healthy people who have HB_sAg in their serum are asymptomatic carriers and are homozygous for the susceptibility gene. We must also assume that we were able to detect all individuals susceptible to becoming carriers. These assumptions are probably not valid in the case of HB_sAg carriers for several reasons. First, although some asymptomatic persons may carry the antigen for many years¹³, some may lose the antigen after a much shorter time¹⁴. Second, pregnant women with clinical hepatitis which does not develop into the chronic carrier state can transmit the infection to their infants who frequently develop chronic antigenaemia¹⁵. Presumably, these infants were not genetically predisposed to becoming carriers but rather became carriers because of their extreme immaturity at the time of infection. Finally, there are some people in all tested populations who show no evidence of having been infected with HBV.

Table 2 Li and Mantel analysis of the distribution of HB_sAg carriers on Santa Cruz

Parental type	No. of children	Estimated	Expected	Standard deviation
Negative $\times$ negative	56	0.167	0.25	0.081
Negative $\times$ positive	26	0.182	0.50	0.123

Given these reservations, however, our analysis of the children of matings between two carriers or between one carrier and one non-carrier rejects the genetic hypothesis, whereas in the same population the analysis of the children of two people who are not carriers of HB_sAg supports it. The importance of these data is not primarily related to the question of genetic predisposition to the HB_sAg carrier state because of serious objections to the validity of the application of segregation analysis to this type of data. This result, however, contradicts several other studies¹⁻⁶ using the same methods. It is possible that in other populations genetic factors may have a more important role, but in Santa Cruz there does not seem to be any simple autosomal recessive gene which significantly affects the distribution of HB_sAg carriers, although there is mimicry of genetic patterns by the offspring of normal parents. Data from Taiwan which were similar to ours were mentioned by Dr Beasley in a discussion at the National Academy of Sciences Hepatitis B Symposium¹⁶. In his study HB_sAg was found in 89% of the children of two positive parents, 55% when mother only was a carrier, 26% when father only was a carrier, and 23% when both parents were negative for HB_sAg.

A significant implication of this result lies in the possibility that chronic HBV infection may be representative of other infections of similar epidemiology with distributions that may also mimic genetic patterns. The observed distribution of carriers is probably the result of the prolonged presence of infectious carriers in combination with limited dissemination regulated by transmission routes involving exposure to infectious blood. Family clustering of antigen carriers may be the result of increased risk of exposure rather than genetically determined susceptibility¹⁷. It is possible that other diseases which are now accepted as genetically determined because of similar distribution patterns may also be caused by infectious agents. One area of particular interest would be that of polygenic birth defects which might be due to *in utero* infection with a virus from an asymptomatic carrier mother rather than the result of a complex set of interacting genes. In addition, late onset diseases which cluster in families may be due to infectious family interactions centred on an asymptomatic carrier rather than genetic predisposition.

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Drugs that disrupt microtubuli do not inhibit lymphocyte activation

LYMPHOCYTE activation by lectins is initiated by the binding of the lectin to a cell surface receptor. It is not known, however, how this event occurring on the cell membrane is converted into a signal that is transmitted to the cell interior. Edelman and coworkers¹⁻⁴ have advanced the hypothesis that lectin receptor sites may be attached to a submembranous microtubular system in a reversible way. This attachment could then determine the receptor mobility in the plane of the membrane and could also constitute a system for transmembrane signal transmission. The hypothesis is based, to a large extent, on the releasing effect that microtubule-disrupting drugs have on the inhibition of surface immunoglobulin cap formation in mouse B lymphocytes by concanavalin A (con A)^{2,3}. Furthermore, evidence has been presented that colchicine delayed and suppressed the appearance of con A-induced blast cells in human lymphocyte cultures, that there is a delay in the onset of DNA synthesis in cultures containing colchicine and that drug sensitivity decreases with increasing culture time in the presence of con A⁴. Cap formation of surface immunoglobulin (Ig) is a phenomenon restricted to Ig-bearing B lymphocytes and these cells are not activated by con A. It is felt therefore that observations on this population do not bear directly on the relationship between the con A receptors and microtubuli as a signal-transducing mechanism. The other observations are suggestive but not conclusive for such a role of the microtubular system.

We have therefore performed experiments to test the hypothesis. If (1) a lymphocyte must have an intact microtubular system to be activated by con A; (2) the drugs disrupt the microtubuli in a reversible way, so that removal results in reassembly; and (3) if lymphocytes are pre-programmed, so that the timing of the sequence of events in a con A-activated cell is fixed, then it must be expected that the presence of microtubule-disrupting agents during the first 24 h of culture only, would result in a delay of the onset and peak of DNA synthesis. It would also be expected that an increase in RNA synthesis induced by con A would be inhibited in the presence of the drugs, since it is compulsory that this early RNA synthesis takes place before DNA synthesis and mitosis^{5,6}.

Table 1 Effect of microtubule-disrupting drugs on rat lymphocyte stimulation

		Thymidine incorporation (48–70 h) as % of control without drug		
Colchicine present in culture		Colchicine concentration (μM)		
0–24 h		0.1	0.5	1.0
24–70 h		109	35	25
48–70 h		6	16	14
		42	27	21
Vinblastine present in culture		Vinblastine concentration (μM)		
0–24 h		0.1	0.5	1.0
24–70 h		120	124	97
48–70 h		120	105	92
		120	105	99
Podophyllotoxin present in culture		Podophyllotoxin concentration (μM)		
0–24 h		0.5	1.0	3.0
24–70 h		101	142	156
48–70 h		95	118	ND*
0–70 h		75	50	58

* ND, not done.

Table 2 Effect of microtubule-disrupting drugs on early RNA synthesis in con A-activated lymphocytes

Addition		Uridine incorporation (% of control)
Colchicine	0.1 μM	96
Colchicine	1.0 μM	99
Vinblastine	0.1 μM	95
Vinblastine	1.0 μM	150
Podophyllotoxin	1 μM	90
Podophyllotoxin	10 μM	117

Rat lymph node cells (2×10^6 per ml) were cultured during 24 h with $5 \mu\text{g ml}^{-1}$ con A. At 24 h, $2 \mu\text{Ci } ^3\text{H}$ -uridine were added; 4 h later cells were collected and processed for counting. Uridine incorporation is expressed as percentage of control

$$\frac{\text{Increase in uridine incorporation with drug}}{\text{Increase in uridine incorporation without drug}} \times 100$$

Experiments were performed with lymph node cells from WAG/Rij rats of 10–14 weeks of age. Cells were cultured at 2×10^6 per ml in Hanks–Eagle's MEM supplied with 10% autologous fresh serum in tightly stoppered glass tubes.

Table 1 shows that with the exception of colchicine at 0.5 and 1.0 μM, none of the three drugs used inhibited thymidine uptake between 48 and 70 h of culture when they were present during the first 24 h of incubation. Colchicine was always strongly inhibitory when added later in the culture period and also when present during the labelling period only. This can be explained by the known inhibitory effect of colchicine on thymidine transport⁷. When colchicine was added at the same time as thymidine for a short labelling period (0.5–2 h), no inhibition of thymidine incorporation was observed. Apparently, the interaction of colchicine with the transport system is a sluggish process.

Vinblastine was not inhibitory at the three concentrations used; it even seemed to stimulate thymidine incorporation somewhat at 0.1 and 0.5 μM. Podophyllotoxin was found to

Fig. 1 Effect of podophyllotoxin on the time course of DNA synthesis in con A-stimulated lymphocytes. Rat lymph node cells (2×10^6 per ml) were incubated at 37 °C in Hanks–Eagle's MEM supplied with 10% autologous serum. Con A ($5 \mu\text{g ml}^{-1}$) and podophyllotoxin were added at $t = 0$. At 24 h the medium was changed (arrow) for one without podophyllotoxin. Cultures were labelled during 2 h with $0.15 \mu\text{Ci } ^{14}\text{C}$ -thymidine (specific activity 50 mCi mmol^{-1}), collected by filtration on glass-fibre filters and the radioactivity counted. All points are the mean of triplicate cultures and data are expressed as counts per min in the presence of con A minus counts per min in the absence of con A. ●, No podophyllotoxin; ▲, 1 μM podophyllotoxin; ■, 5 μM podophyllotoxin.

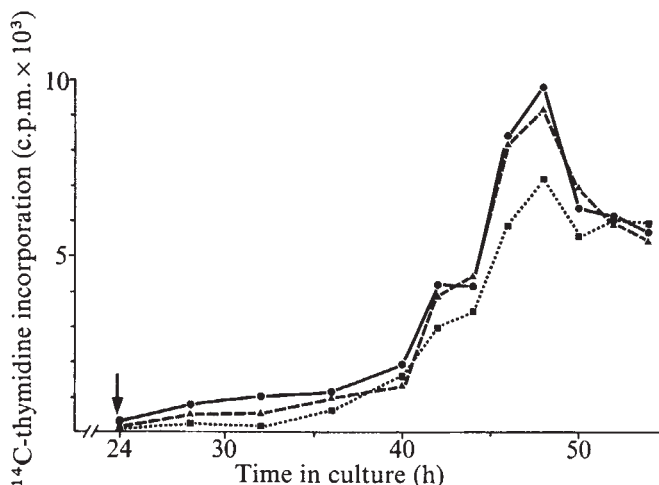


Table 3 Effect of microtubule-disrupting drugs on rat lymphocytes stimulated by con A

Drug	% Blast cells at 48 h	% Blast cells at 72 h	Mitosis per 100 blasts	Viable cells ($\times 10^6$) at 48 h	% Capping
None	37	77	3	1.53	5
Colchicine 1 μ M	29	66	27	1.39	31
Vinblastine 1 μ M	26	69	34	ND*	25
Podophyllotoxin 1 μ M	37	77	26	1.62	16

*ND, not done

Blast cells and mitotic figures were counted in microscopic preparations made according to Sayk and stained with May-Grunwald Giemsa; 200 cells were counted. For the determination of the mitotic index, drugs were added at 40 h of culture and preparations were made 8 h later. Capping was determined as described by Yahara and Edelman³ with FITC-conjugated goat anti-rat immunoglobulin; in the absence of con A, more than 95% of the fluorescent cells capped. Viability was determined according to Stewart and Ingram⁸ with the Coulter counter.

be inhibitory only when present during the entire culture period. More accurate data on the onset and time of peak DNA synthesis were obtained from pulse label experiments. Cells were cultured during 24 h in the presence of the drug and con A. The medium was then removed and replaced by medium without drug. Cultures were subsequently pulse labelled at 2-hourly intervals and left exposed to the label for 2 h. The results of such an experiment with podophyllotoxin is shown in Fig. 1. The drug had no effect on the time of onset, the rate or the peak time of thymidine incorporation. Only the maximum incorporation was somewhat lower with the highest drug concentration. Essentially the same result was obtained with colchicine, except that the rate of increase of thymidine incorporation was slower than in the control during the first 12 h after removal of colchicine. This may be due to a slow release of the inhibition of thymidine transport into the cells.

Table 2 shows that the drugs had no inhibitory effect on the increase of uridine incorporation into RNA in the concentrations used. Table 3 shows that the drugs had the expected effect on surface immunoglobulin cap formation; they partly released the inhibition of anti-Ig induced capping imposed by a high concentration of con A. It also shows that all three drugs blocked mitosis in rat lymph node cells activated by con A. Both observations indicate that the agents can interact with microtubuli in the concentration range used in our system. Table 3 also shows that the drugs do not inhibit blast formation and are not cytotoxic.

Our results indicate that the three drugs in a concentration shown to disrupt the microtubular system do not delay con A-induced DNA synthesis, do not inhibit blast formation and do not inhibit lectin-induced RNA synthesis which precedes DNA synthesis. The results make it unlikely that an intact microtubular system is a strict requirement for signal transmission in lymphocyte activation by con A.

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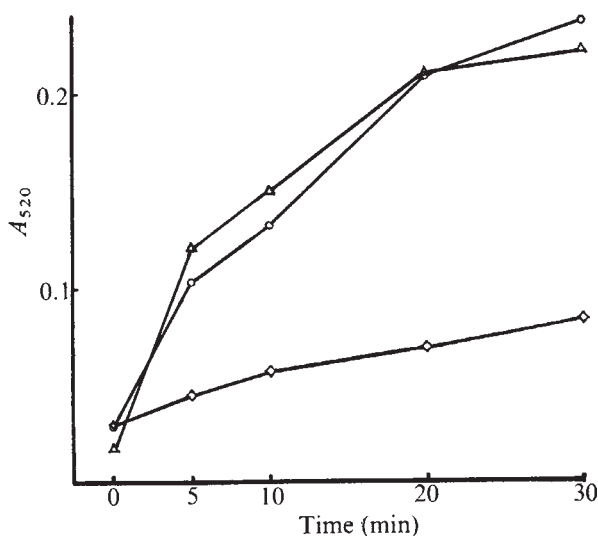
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Synergistic effect of colchicine and cytochalasin D on phagocytosis by peritoneal macrophages

RECENTLY evidence has accumulated for the general involvement of microtubules and microfilaments in several membrane phenomena¹⁻⁸. Usually, low concentrations of colchicine (μ M concentrations) and related reagents have been used as the specific inhibitors for microtubules. In the case of phagocytosis, Ukena and Berlin reported that this reagent did not inhibit phagocytosis *per se* but affected molecular discrimination of membrane proteins during phagocytosis². The mechanism of this interesting phenomenon has, however, not yet been explored.

On the other hand, higher concentrations of colchicine (mM concentrations) have been found to show an entirely different effect on several membrane phenomena. These include several-fold enhancement of Sendai virus-induced fusion of Ehrlich ascites tumour cells and of human

Fig. 1 Time course of ingestion of paraffin oil droplets in the presence and absence of colchicine. The system consisted of 1.7×10^6 cells of mouse peritoneal macrophages suspended in 1.6 ml of Hanks' medium containing colchicine as indicated. After preincubation at 37 °C for 10 min with shaking, ingestion was started by the addition of 0.4 ml of prewarmed paraffin oil emulsion prepared as described in the text. Reaction was stopped by the addition of the medium containing *N*-ethylmaleimide and was assayed as described by Stossel *et al.*¹². ○, No colchicine; △, 5 μ M colchicine; □, 10 mM colchicine.



erythrocytes⁹, and induction of energy-dependent internalisation of the membrane of human erythrocytes^{10,11}. The purpose of this letter is to report the dual effect of colchicine on phagocytosis by mouse peritoneal macrophages and possible cooperation of contractile microfilaments and microtubules in this dynamic membrane reaction.

Phagocytosis of paraffin oil droplets was measured as described by Stossel *et al.*^{12,13} except that Oil Red 5B was used instead of Oil Red O as a paraffin oil soluble dye. As Fig. 1 shows, colchicine had no effect on the rate of phagocytosis at 5 μ M, but at higher concentrations it inhibited the reaction almost completely. The effect of different concentrations of colchicine on this process is summarised and depicted in Fig. 2. The colchicine concentrations required for inhibition of the reaction were similar to those used for stimulation of virus-induced cell fusion⁹ and also for induction of membrane internalisation of human erythrocytes. Primaquine, the other inducer of membrane internalisation¹⁰⁻¹¹, was also effective as an inhibitor of phagocytosis at similar concentrations (Fig. 2). Inhibitory effect of these reagents therefore could not be associated with disruption of microtubules. Cytochalasin D, which interferes with microfilament function, is a potent inhibitor of phagocytosis at the same concentrations which inhibit actomyosin ATPase¹⁴ and virus-induced cell fusion⁹ (Fig. 3a). Effective concentrations of this reagent were found to be lower than those of cytochalasin B. Furthermore, the former is more specific than the latter: cytochalasin B both inhibits the function of microfilaments and facilitates diffusion of sugars through plasma membranes¹⁵, whereas cytochalasin D is not inhibitory for membrane transport⁹. Accordingly, the participation of microfilaments in phagocytosis reported earlier on the basis of the inhibitory effects of cytochalasin B (ref. 16) is confirmed by the present experiments.

Furthermore, as depicted in Fig. 3a, low concentrations of colchicine were found to inhibit the reaction synergistically with suboptimal concentrations of cytochalasin D. A similar cooperative effect could be obtained with vinblastine instead of colchicine at concentrations which were reported to disrupt microtubules¹⁷ (Fig. 3b). The effect of reagents which inhibit microtubules and microfilaments on phagocytosis is thus closely similar to those on capping of the surface immunoglobulin of lymphocytes⁴⁻⁷. This cooperative effect of inhibitors of microfilaments and microtubules may be explained by an increase in sensitivity of microfilaments component(s) to cytochalasins in the presence of microtubule inhibitors. Similar modulation of cytochalasin-sensitivity by extracellular Ca^{2+} has been found in the case of virus-induced cell fusion¹⁸. On the other hand, primaquine which behaved similarly with colchicine at millimolar concentrations did not mimic the effect of the latter at

Fig. 2 Effects of colchicine or primaquine on the rate of ingestion of paraffin oil droplets. The system and conditions were similar to those described in Fig. 1 except that 3.8×10^6 cells were used and primaquine was employed instead of colchicine when indicated. Duration of the ingestion was 20 min. ●, Colchicine; ○, primaquine.

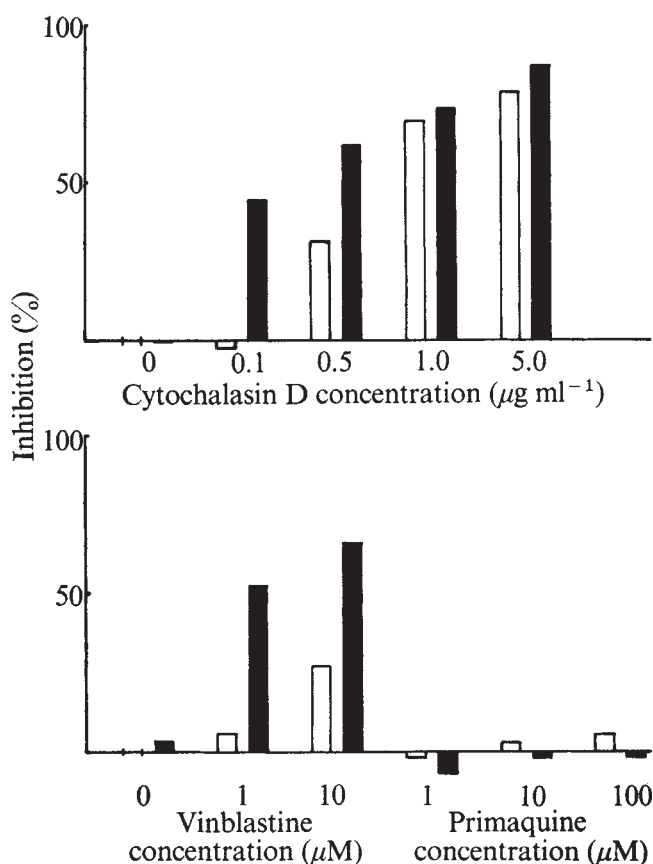
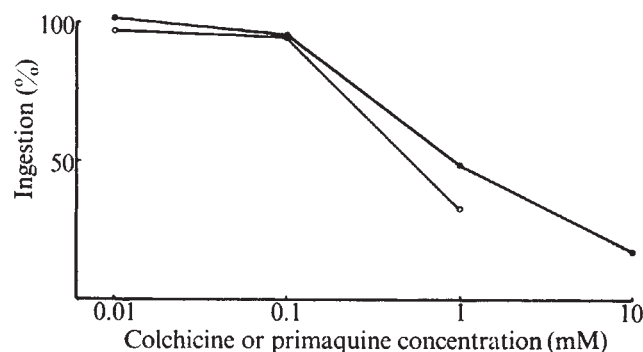


Fig. 3 Cooperative inhibition of phagocytosis with a, cytochalasin D and colchicine or b, cytochalasin D and vinblastine or primaquine. The system and conditions were similar to those described in Fig. 1 except that 3.6×10^6 and 9.5×10^6 cells were used in a and in b, respectively, and effectors were included in the system as indicated. Dimethylsulphoxide which was used for solvent of cytochalasin D was included in all tubes to a final concentration of 1% (v/v). Each bar represents the mean of 3-4 experiments. a, Open bar, cytochalasin D only; solid bar, cytochalasin D plus 10 μM colchicine; b, Open bar, either vinblastine or primaquine only; solid bar, 0.1 $\mu\text{g ml}^{-1}$ of cytochalasin D plus either vinblastine or primaquine as indicated.

micromolar concentrations, in other words, no cooperativity was found between primaquine and cytochalasin D (Fig. 3b).

From the results described above, it could be concluded that these cytoskeleton components may name an important part in phagocytosis by mouse peritoneal macrophages. The possibility of cooperation of these components in cap formation has been reported before⁴, and their cooperative participation in concanavalin A-induced agglutination of 3T3 cells has recently been reported⁸. Thus, membrane-cytoskeleton interaction seems to have some ubiquitous role in dynamic properties of biomembranes. Furthermore, it may be reasonable to assume that high concentrations of colchicine and/or primaquine influence phagocytosis by affecting some component(s) other than microtubules. Since similar concentrations of these reagents are known to affect the other membrane phenomena these effects of colchicine and primaquine are of considerable interest.

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Nuclear magnetic resonance studies of the adrenal gland and some other organs

KNOWLEDGE of the state of many chemicals in cell compartments is based on inference from analytical methods carried out after partial or complete disruption of the cell. This situation arises as most of the cell contents have similar spectroscopic properties and cannot be studied inside a cell. We have shown that the contents of intact vesicles of the adrenal medulla (chromaffin granules) can be studied by nuclear magnetic resonance (NMR) in separated vesicles¹. (We have subsequently shown (unpublished observations) that the spectra are quantitative and that all the adrenaline present in the granules is observed by NMR spectroscopy.) From this study¹ we could establish an outline structure of the internal solution of these granules. The question then arises as to whether precisely this structure exists in the organelle within the cell or whether isolation of organelles such as these modified their properties. We report here that improved NMR methods can provide detailed information about the environment and mobility of small molecules and some proteins even within intact organs. As little as 10 mg (dry weight) of tissue is required. No other technique can provide such information at present. Furthermore, the NMR data can be used to describe structural features of the organisation of the cell.

The possibility of observing molecular species differentially by NMR spectroscopy arises from the following considerations. Nuclear magnetic resonance spectra of large aggregates of small molecules (for example, lipids in membranes) or of very large single molecules (for example, proteins of molecular weight greater than about 50,000) usually consist of broad, ill-defined absorptions over wide spectral regions, even at high magnetic fields. By way of contrast, highly mobile molecules (or parts of molecules) give rise to sharp proton resonances. Using convolution difference spectroscopy², we have shown that sharp lines can be separated from broad lines and we have used this method in the work described here. In addition, new methods of spectral simplification which depend on the use of T_1 and T_2 pulse sequences³ allow separation of resonances which are undergoing different rates of nuclear magnetic relaxation, that is, sharp and broad resonances. In this way the narrow lines arising from rapidly tumbling molecules are separated from the broad resonances of the slowly tumbling molecules. It must be stressed that only resonances of highly mobile units are observed in the NMR spectrum in this work.

A typical NMR spectrum of the rat adrenal medulla from which the adherent inner cortex (the reticularis zone) has been removed is shown in Fig. 1a. This should be compared with the spectrum obtained from slices of bovine medulla (Fig. 1b). A similar spectrum is also obtained with slices of

adrenal medulla from pig or sheep. Resonances which can be distinguished at 8.30, 7.95 and 5.89 p.p.m. are those of ATP H_8 , H_2 and H_1 , protons respectively while resonances at 6.78 (broad), 3.10 and 2.69 p.p.m. arise from the adrenaline aromatic, $-CH_2-$ and $-CH_3$ protons respectively. These

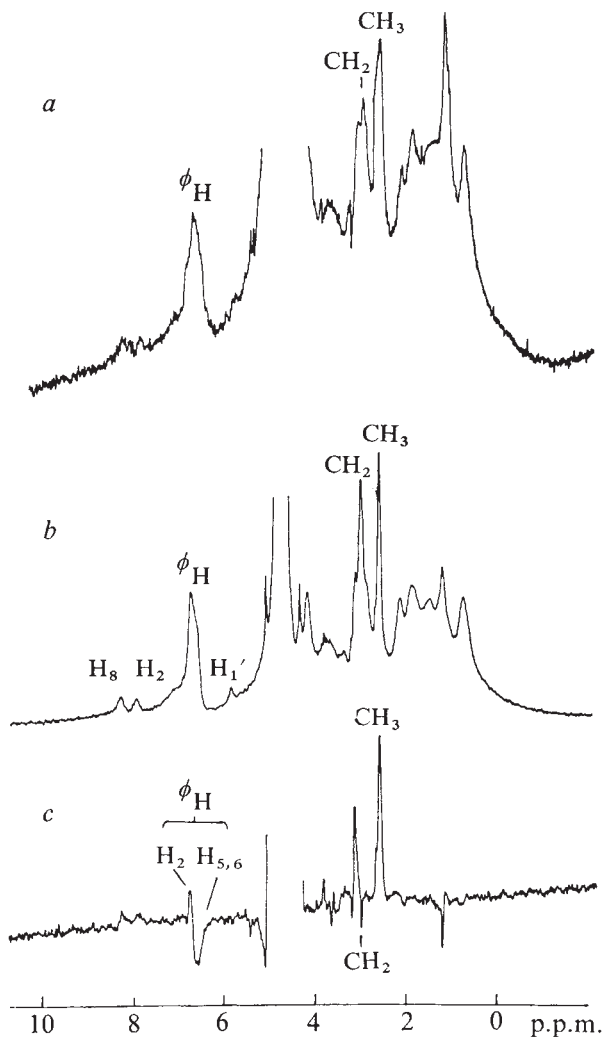


Fig. 1 Nuclear magnetic resonance spectra of dissected rat adrenal medulla, (a) bovine adrenal medulla slice (b) and bovine medulla slice (c) using a $(90^\circ-\tau-180^\circ-\tau-\text{echo})_n$ pulse sequence. $\tau = 60$ ms. This pulse sequence separates resonances according to their respective transverse relaxation times, T_2 . The component of magnetisation perpendicular to the applied magnetic field decays to zero during the delay time τ for resonances (of the macromolecule) with very short T_2 . Consequently no peak (or only a very small peak) is observed for these resonances in the final spectrum. On the other hand, the resonances of the small, highly mobile molecule, adrenaline, are characterised by long T_2 and retain much of their original intensity when observed after the delay time τ . Thus the adrenaline resonances are observed in the resulting spectrum while resonances of the macromolecular systems are reduced greatly in intensity or completely removed. The doublet resonances of the adrenaline $-CH_2-$ group and the aromatic H_5 and H_6 protons are inverted at the value of τ (60 ms) in the Fig. 1c due to modulation of the spin coupling³. Spectra were recorded at 19°C for a and 23°C for b and c using a Bruker 270 MHz spectrometer with an Oxford Instrument Company magnet. TSS was used as an internal standard. The adrenals were dissected from rats immediately after death and the cortex was separated carefully from the medulla by dissection under the microscope. The cortex and medulla were washed separately several times in a medium containing NaCl (160 mM), KCl (5 mM), $CaCl_2$ (2.2 mM) PIPES (2.5 mM), glucose (5 mM) and iproniazid (0.1 mM) in D_2O at pH 7.1. The spectrum of the isolated rat medulla (7 mg dry weight) was recorded using a Wilmad spherical NMR microcell assembly. Slices of bovine adrenal medulla were obtained from fresh adrenal glands after careful removal of the cortex and washed with the medium used for the rat medulla.

resonance positions are exactly those seen in the spectrum of isolated chromaffin granules¹, the peaks being shifted upfield with respect to the positions of resonances of the molecules in free solution. The resonances of adrenaline can be extremely effectively resolved by convolution difference spectroscopy or using $(180^\circ-\tau-90^\circ)_n$ (T_1) and $(90^\circ-\tau-180^\circ-\tau-\text{echo})_n$ (T_2) pulse sequences (Fig. 1c). When the slices are aged or incubated at about 50 °C, some of the adrenaline is released from the medulla and sharp resonances from free adrenaline $-\text{CH}_2$ and $-\text{CH}_3$ protons are observed at 3.21 and 2.77 p.p.m. respectively, with the aromatic nuclear resonances at 6.87, 6.93 and 6.95 p.p.m. These results confirm that the same adrenaline-ATP complex exists in the intact adrenal medulla as found in isolated chromaffin granules¹.

The peaks upfield from 2.5 p.p.m. in the spectrum of the beef medulla correspond to the protein chromogranin A, which is clearly in the same mobile, random coil state in the intact adrenal medulla as in the isolated vesicles¹. The peak near 1.3 p.p.m. is, however, slightly more intense than is predicted for the amino acid composition of chromogranin, and this extra absorption probably reflects some small contribution from lipid fatty acid chain $-\text{CH}_2-$ groups. A shoulder near 3.2 p.p.m. is then likely to arise from the choline $\text{N}(\text{CH}_3)_3^+$ head group of a lecithin lipid. In spite of the very high lipid content of the adrenal medulla, no resonances of lipid protons are observed except for the two very weak signals mentioned above. Thus the medulla lipids must be in a state of slow molecular motion in which there is incomplete averaging of the dipolar interactions between spins resulting in severe broadening of the NMR resonances⁴. Further evidence for motional restraints of the lipid hydrocarbon chains is obtained from natural abundance ^{13}C NMR spectra of slices of medulla from pig or beef (our work in preparation) in which the chain methylene resonances are resolved only as a very broad featureless band. A sharper ^{13}C resonance arising from the choline $\text{N}(\text{CH}_3)_3^+$ group suggests greater mobility of at least some of the lipid headgroups. To investigate further the apparent rigidity of the membranes comprising the bovine adrenal medulla, ^1H NMR spectra were obtained for isolated chromaffin granule membrane, plasma membrane, mitochondrial membrane and microsomal membrane preparations. In each case a high resolution NMR spectrum was not observed although the resolution of a greatly broadened $\text{N}(\text{CH}_3)_3^+$ peak again suggested limited motions of the choline head-group. Our results are in accord with those of previous NMR investigations of other biological membranes^{4,5}, which also demonstrated a state of slow molecular motion of the component lipids.

The ^1H NMR spectrum of rat adrenal cortex (predominantly the fasciculata and reticularis zones) obtained by dissection, under the microscope, of the rat adrenal gland after removal of the elastic capsule and adherent outer cortex (glomerulosa zone), is shown in Fig. 2a. The well resolved resonances are those of highly mobile lipid molecules. The observed lipid contains very little, if any, lecithin because no sharp choline $\text{N}(\text{CH}_3)_3^+$ proton resonance (usually the sharpest due to the high mobility of this group⁶) is observed. The high intensity of the $\text{CH}=\text{CH}$ resonance at 5.33 p.p.m. and the relatively low intensity of the $-\text{CH}_2-$ peak at 1.30 p.p.m., show that highly unsaturated lipid is being observed. Such polyunsaturated fatty acids are known to comprise a sizeable proportion (about 30%) of rat adrenal cholesterol esters⁸, and we conclude that the lipid signals in the NMR spectrum arise from lipid droplets, known to be abundant in the fasciculata (and glomerulosa) zone of the rat adrenal cortex⁷. (It should also be noted that, in intact plant seeds, we have obtained high resolution ^{13}C spectra (unpublished observations) from the lipid droplets which occur as cytoplasmic stores of fats and oils⁹.) Although 5% of the wet weight of the rat adrenal cortex is cholesterol, about 75% of which is stored within the lipid

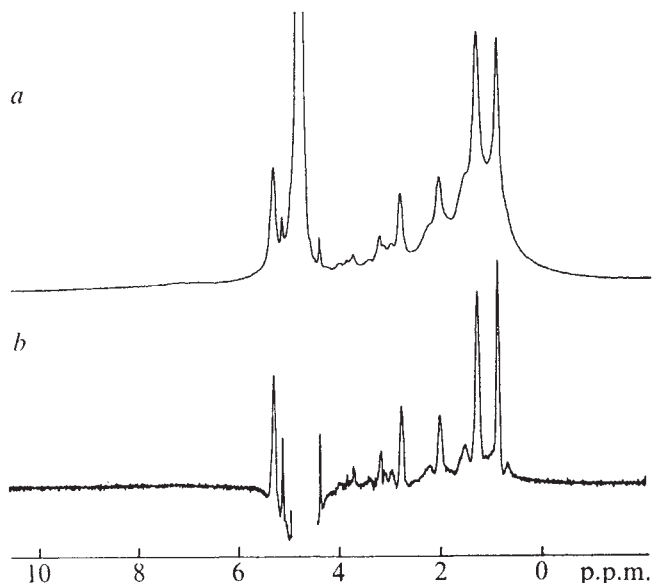


Fig. 2 NMR spectra of rat adrenal cortex (predominantly the fasciculata and reticularis zones). *a*, Normal FT spectrum; *b*, convolution difference spectrum showing a peak at 0.70 p.p.m. assigned to the 18-methyl group of cholesterol.

droplets⁹, no well resolved cholesterol resonances are observed in the NMR spectrum. When the convolution difference technique² is used, however, a weak signal is resolved at 0.70 p.p.m., which probably arises from the cholesterol 18-methyl group (Fig. 2b). This signal is too weak to account for all the cholesterol present in the liposomes—the remainder of the cholesterol must therefore be immobilised such that its NMR resonances are too broad to be observed. We note that cholesterol proton resonances are also absent from the NMR spectra of cholesterol-lipid systems¹⁰.

We now consider the advantages and new information which we have obtained from our ability to study the NMR spectra of whole organs. First, our observation of the NMR spectrum of the internal contents of the granules enables us to state that the dynamic structure of these granules, as far as ATP, adrenaline and the protein chromogranin are concerned, is the same in the whole organ as in isolated vesicles. Furthermore, this same structure is conserved in the adrenal medulla from four species. No other technique could lead to this conclusion. Second, evidence is obtained for the immobility of the lipid components of the adrenal medulla, in contrast to the state of rapid molecular motion of certain lipids in the rat cortex. Third, although the cortex is a storage organ for sterols much as the adrenal medulla is a store of adrenaline, the two types of store are in totally different physical states. Finally, the NMR technique is readily applicable to other intact biological tissues such as homogeneous cell cultures, and we have obtained well-resolved spectra from splenic nerve and blood platelets.

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Enzyme replacement therapy by fibroblast transplantation in a case of Hunter syndrome

SUPPLEMENTATION of deficient enzymes essential for complete catabolism of glycosaminoglycans (GAG) has been used with limited success in several types of mucopolysaccharidosis¹⁻⁴. The beneficial effects and concomitant changes in urinary GAG after this form of treatment, however, have been only transient, presumably because of the short life *in vivo* of the enzymes involved⁵⁻⁷. Because of this limitation, we recently tried, by means of skin transplantation, to provide a more permanent source of corrective enzymes in a patient with Hunter syndrome⁸. Although two HLA antigens from each donor were incompatible with those of the patient and both grafts had been visibly rejected within 3 months, there was a marked increase in breakdown and excretion of GAG subsequent to treatment, which lasted for more than 9 months. In addition, the activity of Hunter corrective enzyme isolated from the patient's urine, was also significantly increased. We attributed the effectiveness of the skin transplant to the release of Hunter corrective factor by donor cells and its uptake by host cells, in a manner analogous to that described for fibroblasts *in vitro*⁹⁻¹¹. We have now attempted to increase further both the effectiveness and longevity of replacement therapy, using fully histocompatible skin fibroblasts injected subcutaneously as a source of corrective enzyme. An advantage of this procedure is that surgery is not required and it would in principle be applicable to other genetic deficiency diseases of lysosomal enzymes.

The patient chosen for this attempt was a 9-yr-old male

Fig. 1 Total 24-h excretion of urinary uronic acid before and after implantation of fibroblasts. Cross hatched areas of columns, polymeric uronic acid^{13,14}, open areas, oligosaccharides. The uronic acid contents of all 24-h collections were calculated and the values depicted were selected as representative. Values for two age-matched normal subjects were: polymeric GAG per 24 h, 3.5 mg and 6.3 mg and oligosaccharide, 85.9 mg and 75.6 mg. The day on which fibroblasts were implanted is designated day 0 and the days before implantation by negative numbers.

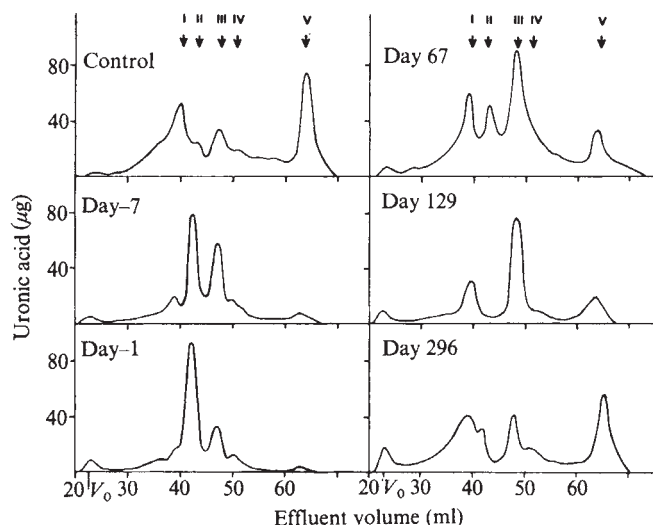
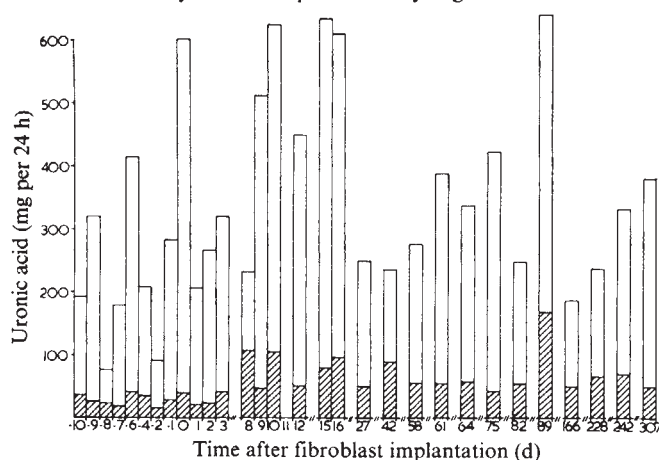


Fig. 2 BioGel P-2 filtration profiles of uronic acid oligosaccharides eluted from Dowex-1. Samples containing about 1 mg of uronic acid from each 24-h collection depicted in Fig. 1 were chromatographed on a column (13 mm × 600 mm) of BioGel P-2, 200-400 mesh packed in and eluted with 0.2 M sodium acetate, pH 6.8. Fractions of 0.6 ml were collected. All samples were subjected to gel chromatography and the profiles shown were selected as representative of the changes observed and were unaffected on days of high (89) or lower (82) uronic acid output.

(N.G.) in whom the diagnosis of Hunter syndrome was suspected on clinical features¹² and confirmed by correction studies of cultured fibroblasts using typed reference cells.

The patient and his sister, who was the donor, had identical HLA antigens, ABO and Rh blood groups (HLA: 2, 3, 12, W5; group O, Rh-ve). No reaction was detected when leukocytes from the patient and donor were mixed. The sister may have been a heterozygote (possibility 1 in 2).

The accelerated rate of incorporation of labelled sulphate by the patient's cultured fibroblasts before transplantation was corrected by culture of his cells in medium previously used to culture his sister's fibroblasts¹⁰. Normal donor fibroblasts were grown *in vitro* from a punch biopsy of skin taken from the sister. A suspension containing 2.2×10^8 cells was implanted subcutaneously into the patient in three dorsal sites. To minimise the risk of graft rejection, immunosuppressive therapy (Azothioprine, 25 mg daily and prednisolone, 50 mg daily) was begun 10 d before implantation, continued in the same dose for 6 months and then reduced progressively during a further 9 months.

Polymeric GAGs were precipitated^{13,14} from aliquots of 24-h urine collections, taken from the patient before and after implantation and from a 10-yr-old normal female control. The oligosaccharides remaining in the supernatant were collected on a column of 'Dowex' 1 × 2 (Cl⁻ form) washed with water, and then eluted with 2 M HCl and immediately freeze dried. Uronic acid contents were determined by an automated method¹⁵. Hunter corrective factor was isolated from the remaining volume of each urine collection and units of correction calculated as described before⁸.

On the day fibroblasts were implanted the excretion of uronic acid increased to a level approximately double the preimplantation mean and to about 50% above the highest preimplantation value recorded. We attributed this immediate increase to Hunter corrective factor released by the fibroblasts into the medium in which they had been suspended before injection. The amount of uronic acid excreted per 24 h then fell and remained near preimplantation levels for several days, before increasing above pre-treatment values (Fig. 1). This cyclical pattern was repeated several times during the period monitored but the overall

Table 1 Changes in percentage distribution of oligosaccharides eluted from a representative selection of BioGel P-2 chromatographs before and after implantation

Day of treatment	% Total uronic acid eluted in each component separated				
	I	II	III	IV	V
-7	13.5	41.6	29.0	12.2	3.7
-6	19.4	20.7	48.0	6.2	5.7
-1	15.7	55.1	17.2	10.0	2.0
+58	15.1	13.4	14.7	20.1	36.7
+89	16.9	5.3	14.5	43.2	20.2
+228	17.3	7.8	24.5	19.9	30.5
+296	33.8	8.0	15.4	7.6	35.2
Normal urine	36.7	6.2	14.8	9.9	32.2

Fractions I to V were numbered in order of decreasing hydrodynamic size as described in the text.

output of uronic acid was on average 50% greater than before implantation. Moreover, the polymeric component approximately doubled from the eighth day after implantation (Fig. 1).

When chromatographed on BioGel P-2 the oligosaccharides in normal urine separated into three major and two minor components (Fig. 2). The positions where the three principal components eluted in order of decreasing hydrodynamic size were: I, equivalent to the tetrasaccharides of chondroitin sulphate produced by the action of hyaluronidase (EC 3.2.1.35); II, equivalent to the monosulphated unsaturated disaccharide produced by the action on chondroitin-4-sulphate of Chondroitinase ABC (EC 4.2.2.4) (Miles); V, coincident with glucuronolactone. Although the oligosaccharides in the patient's urine were eluted at positions identical with those from normal urine (Fig. 2) before treatment, their relative proportions differed markedly (Table 1). Component II accounted for 20.7–55.1% of the total uronic acid eluted from the column, whereas in normal urine it represented only 6.2%. Likewise, the relative amount of component III was more than twice that in normal urine but the monosaccharide (component V) was less than 10% of that normally present. In addition a minor fraction comprising larger unresolved oligosaccharides was eluted with the void volume in samples prepared from Hunter urines.

After implantation, there was a progressive decrease in the relative proportions of components II and III (Table 1) and an increase in component V, until the overall elution profiles of the oligosaccharides were almost identical with those of normal urine (Fig. 2). The glucuronic/iduronic acid ratios of combined fractions II and III showed that more than 20% of the total uronic acid in these fractions was present as iduronic acid before treatment, whereas iduronic acid accounted for less than 10% some 2 months after implantation. At the same time the iduronate content of the monosaccharide fraction V increased (Table 2).

The activities of Hunter corrective factors isolated from the patient and from age-matched normal urines were calculated from their ability to correct abnormal incorporation of labelled sulphate by the patient's cells⁸. Four healthy

Table 2 Changes in relative proportions of iduronic acid present in oligosaccharides eluted from BioGel P-2 before and after fibroblast implantation

Day of treatment	Iduronate as % of total uronic acid	
	Fraction II+III	Fraction V
-7	27.8	19.8
-4	19.7	—
-1	21.7	17.6
+58	10.4	18.1
+65	8.0	27.5

Combined fractions II and III and fraction V were analysed by gas liquid chromatography as their trimethylsilyl derivatives after methanolysis¹⁰. Iduronic acid was calculated as a percentage of the total uronic acid detected.

boys aged 4–11 yr excreted $29.7\text{--}67.7 \pm 18.8$ U (mean 45.1 U) of corrective factor per 24 h, whereas the patient excreted 0.0–1.04 U per 24 h (mean 0.47, $n=4$) before implantation. After implantation the excretion increased to 6.7% of normal values, with an average of 3.0 ± 1.7 U per 24 h ($n=15$).

In the severe form of Hunter syndrome, mental and physical deterioration progress relentlessly, with death often in the second decade. In our patient there was a marked improvement in general behaviour with loss of aggressive tendencies and no detectable deterioration in intellectual function during 10 months. His liver size diminished considerably but splenomegaly persisted. Full clinical assessment will be reported after a longer period of study.

Before replacement therapy can be effective, the level of deficient enzyme (sulpho-L-iduronate sulphatase) must be increased significantly and this increase must be sufficient to breakdown incompletely degraded heparan and dermatan sulphates stored within the tissues. All the changes that occurred after implantation, which were maintained many months afterwards, provide strong evidence that the corrective factor produced by the normal fibroblasts had enabled a significant proportion of the accumulated GAG and oligosaccharides within the tissues to be broken down to completion. Immunosuppressive drugs given before transplantation did not increase output of uronic acid and when gradually reduced the average excretion of uronic acid remained high.

The precise mechanism of action of the donor fibroblasts is as yet unknown, but it is assumed that they can release enough Hunter corrective enzyme to induce the observed effects when taken up by the host cells, as has been demonstrated for fibroblasts *in vitro*^{8–11}. In Sanfilippo B syndrome a 2–5% increase in the intracellular level of α -N-acetylglucosaminidase can induce 42–70% correction in abnormal GAG metabolism *in vitro*⁸. If the same were true for Hunter corrective factor the increase in its activity observed after treatment might be sufficient to induce a large increase in catabolism of the accumulated GAG. Nevertheless, that so few normal cells should have an effect when transplanted is remarkable, but a small graft of skin in another Hunter patient⁸ had a similar effect. It is possible that the active enzyme survives for longer than normal in the host. In the environment of the host, production of enzyme by normal cells may also be fully derepressed.

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Small molecular weight β_1 serum protein which specifically inhibits human collagenases

NEUTRAL collagenases are specific collagen-degrading enzymes which can be identified and isolated from the media of a wide variety of human tissues in culture. *In vivo* the enzymes function extracellularly at neutral pH and probably have a crucial role in the catabolism of collagen. Evidence for the involvement of the enzymes in diseases characterised by disordered collagen metabolism is substantial but our understanding of the mechanism of regulation of extracellular collagenase activity is poor. Since the specific inhibition of collagenase might offer a therapeutic approach to some connective tissue diseases we have attempted to identify the natural inhibitors of this enzyme in human serum. We report here our finding of a β_1 serum protein which specifically inhibits human collagenase. Because of its small molecular size (molecular weight about 40,000) this inhibitor may be important in the regulation of tissue collagenase activity.

It has been known for some time that whole serum can inhibit human collagenase activity derived from a wide variety of tissues¹⁻³. This action is largely attributable to the α_2 -macroglobulin component⁴⁻⁷ which also binds and inhibits many other less specific proteolytic enzymes⁸. Because of its high molecular weight (725,000, ref. 9) this antiproteinase is probably excluded from many tissue sites by permeability barriers and thus the role of smaller anti-proteinases may be more important in the control of enzyme activities within tissues. One such inhibitor which has been the subject of considerable study is α_1 -antitrypsin, molecular weight 45,000, but the capacity of this serum protein to inhibit neutral collagenases has been in doubt^{3,5,6,10,11}.

We examined the inhibitory capacity of the small serum proteins against human collagenases and found that although α_1 antitrypsin was non-inhibitory a slightly smaller protein in low concentrations was capable of inhibiting collagenase activity derived from several different tissues. This serum protein of molecular weight ~ 40,000 had no effect on the activity of trypsin or papain, suggesting a certain degree of specificity. The finding of a natural inhibitor of collagenase activity, with a molecular size similar to that reported for some collagenases themselves, suggested that it might function physiologically as a regulatory factor in collagen catabolism^{3,12}.

The small collagenase inhibitor has been partially purified and separated from the well known serum antiproteinases α_2 macroglobulin and α_1 antitrypsin by a procedure involving gel filtration in Sephadex G-200 and G-100 superfine, with an intermediate ion exchange chromatography step using DEAE-Sephadex³. This preparation, when examined by Agarose electrophoresis, was shown to migrate as a

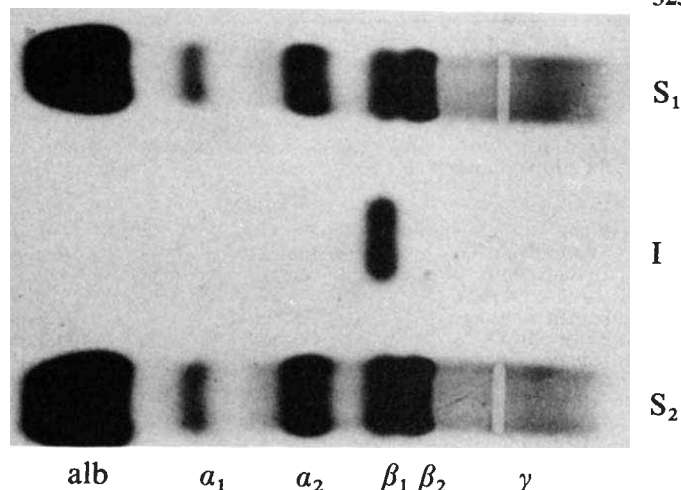


Fig. 1 Electrophoresis of the small collagenase inhibitor on Agarose. The inhibitor preparation and samples of normal human serum were applied to an Agarose film (Corning ACI) and subjected to electrophoresis for 1 h at 3 mA using 50 mM sodium barbital buffer (pH 8.6). The protein bands were stained with Coomassie brilliant blue. I, collagenase inhibitor; S₁ and S₂, serum samples.

single protein band corresponding to the position of a β_1 serum protein (Fig. 1). We therefore propose to call the inhibitor β_1 anticollagenase (β_1 -AC). To establish the specificity of β_1 -AC partially purified preparations together with purified samples of α_2 macroglobulin (α_2 -M) and α_1 antitrypsin (α_1 -AT) were examined for their action against rheumatoid synovial and other human collagenases, and against enzymes representative of the principal classes of proteinases.

At 25 °C, neutral pH and in the presence of calcium ions, human collagenases attack collagen molecules in solution at a single specific locus producing two products which represent three-quarter and one-quarter length fragments of the molecule^{1,4}. This reaction can be monitored by viscometry and a final drop in specific viscosity of a collagen solution of approximately 55-58% usually indicates that the reaction is complete. Figure 2 shows that the addition of β_1 -AC to a reaction mixture of human synovial collagenase and collagen significantly reduces the rate of

Fig. 2 Effect of β_1 -AC on the reaction between rheumatoid synovial collagenase and collagen in solution determined by viscometry at 25 °C. Samples (25 μ l) of synovial enzyme were added to 100- μ l samples of β_1 -AC (2 mg ml⁻¹) and bovine serum albumin (2 mg ml⁻¹). 100 μ l of each sample was then added to separate 1 ml capacity Ostwald viscometers each containing 0.9 ml of a collagen solution. The final reaction mixtures of 1 ml contained: 0.4 M NaCl, 50 mM Tris-HCl buffer (pH 8.0) 10 mM CaCl₂, 0.05% arginine, 0.7 mg collagen and collagenase, with added β_1 -AC protein (○) or with added bovine serum albumin as control (●). The outflow time for water at 25 °C was 28 s for both viscometers.

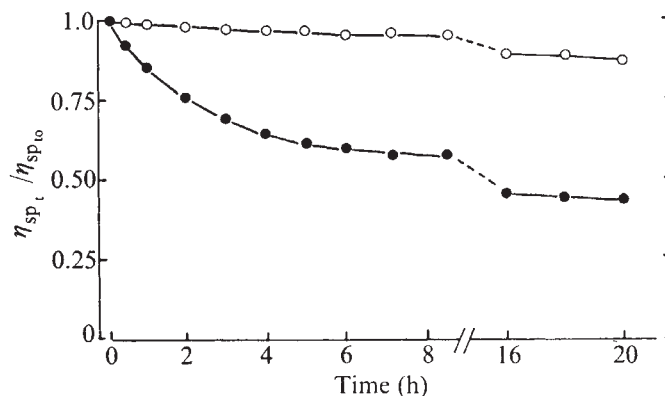


Table 1 The effect of β_1 -AC on the enzymatic activity of human collagenases and various proteinases

Enzyme	Proteinase Type	Substrate	Enzyme activity		% Inhibition
			Control	+ β_1 -AC	
Trypsin	Serine	Casein*	1.68 (26)	1.74	0
Subtilopeptidase A	Serine	Casein	1.01 (16)	0.95	5
Elastase	Serine	Elastin-orcein†	0.19 (35)	0.20	0
Papain	Thiol	Casein‡	0.43 (7)	0.43	0
Ficin	Thiol	Casein	1.39 (22)	1.33	4
Thermolysin	Metal	Azocasein§	1.44 (22)	1.46	0
Snake venom (<i>Crotalus atrox</i>)	Metal	Azocoll	0.45 (18)	0.47	0
Pepsin	Carboxyl	Haemoglobin¶	0.58 (25)	0.60	0
Cathepsin D	Carboxyl	Haemoglobin**	0.32	0.30	6
<i>Human Collagenases</i>					
Gastric mucosal	Metal	Collagen††	1,200 (40)	240	80
Rheumatoid synovial	Metal	Collagen	1,220 (41)	731	40
Skin	Metal	Collagen	1,150 (38)	644	44
Granulocyte	Metal	Collagen	1,090 (36)	872	20

With each series of assays, control incubations containing twice the amount of enzyme were included to ensure that reactions were terminated at a point where they were still linear. Figures in parentheses shown after the control enzyme activities indicate the proportion of substrate degraded as a percentage of total available substrate determined by complete enzymatic digestion. Results presented are the means of duplicate assays after subtracting control values and are expressed as percentage inhibition of the control. *10 mg of casein dissolved in 2 ml 100 mM Sorenson's phosphate buffer (pH 8.0) containing 0.2 μ g trypsin (Sigma, Type III) or 0.2 μ g subtilopeptidase A (Sigma) was incubated for 16 h at 37 °C with and without the β_1 -AC protein (40 μ g ml⁻¹). Each reaction was terminated by the addition of cold 10% trichloroacetic acid, centrifuged for 20 min at 3,000g, and the absorbance of the supernatant measured at 280 nm. †2 mg of elastin-orcein (Sigma) suspended in 2 ml 100 mM Tris-HCl buffer (pH 8.8) containing 2 μ g elastase (Sigma, Type III) incubated for 16 h at 37 °C with and without the β_1 -AC protein (40 μ g ml⁻¹). The reaction mixtures were filtered and the absorbance of the filtrates measured at 590 nm. ‡10 mg of casein dissolved in 2 ml 100 mM Sorenson's phosphate buffer (pH 8.0) containing 50 mM cysteine, 10 mM EDTA, 1 μ g of papain or ficin (Sigma) was incubated for 16 h at 37 °C with and without β_1 -AC protein (40 μ g ml⁻¹). The reactions were terminated and measured as described for (*). §10 mg of Azocasein (Sigma) in 2 ml 100 mM Tris-HCl buffer (pH 8.0) containing 10 mM CaCl₂ and 0.2 μ g thermolysin (Sigma protease, Type X) was incubated for 16 h at 37 °C with and without β_1 -AC protein (40 μ g ml⁻¹). The reactions were terminated as described in (*) and the absorbance of the supernatants measured at 366 nm. ||5 mg of Azocoll (Calbiochem) in 2 ml 100 mM Tris-HCl buffer (pH 8.0) containing 10 mM CaCl₂ and 2 μ g *Crotalus atrox* venom (Sigma) were incubated for 16 h at 37 °C with and without β_1 -AC protein (40 μ g ml⁻¹). The reaction mixtures were filtered and the absorbance of the filtrates measured at 520 nm.

¶5 mg of haemoglobin (Sigma) dissolved in 2 ml 100 mM sodium acetate buffer (pH 3.2) containing 0.2 μ g pepsin were incubated for 16 h at 37 °C with and without β_1 -AC (40 μ g ml⁻¹). The reactions were terminated and measured as described for (*). **This assay was kindly undertaken by Dr Alan Barratt of the Strangeways Research Laboratory, Cambridge, using haemoglobin as substrate¹⁴. ††Collagenase activity was assayed by measuring the release of soluble radioactive products from a pellet of thermally reconstituted ¹⁴C-glycine-labelled collagen fibrils¹². Each reaction mixture contained 3,000 c.p.m. and the assays were incubated for 16 h at 37 °C with and without β_1 -AC (40 μ g ml⁻¹).

collagen cleavage when compared with that of the control reaction.

For comparison, the effect of purified α_2 -M and α_1 -AT on synovial collagenase activity was also examined by viscometry and only the former was found to be inhibitory. These results confirmed previous data obtained from collagenase assays at 37 °C using thermally reconstituted ¹⁴C-labelled collagen fibrils¹² indicating that the two serum proteins β_1 -AC and α_2 -M are potent inhibitors of human collagenase activity whereas α_1 -AT is non-inhibitory.

As earlier work had shown the β_1 -AC protein to have no inhibitory effect on trypsin and papain³, studies were undertaken using nine different enzymes selected to represent the serine, thiol, metal-dependent and carboxyl proteinases, in order to obtain further information on the specificity of the inhibitor (Table 1). The serine proteinases trypsin, subtilopeptidase A and elastase, and the thiol proteinases papain and ficin were not inhibited by β_1 -AC, but all were susceptible to inhibition by α_2 -M. Since human collagenases are characterised as metal-dependent proteinases it was of particular interest to see if other examples of this group of enzymes were similarly inhibited by β_1 -AC. Thermolysin and the snake venom from *Crotalus atrox* were selected as examples of metal-dependent proteinases but neither was inhibited by β_1 -AC. The addition of β_1 -AC to cathepsin D and pepsin, which are both carboxyl or acid proteinases, also failed to produce any significant inhibition.

In summary, the β_1 -AC protein seems to be a specific inhibitor for human neutral collagenases such as those derived from rheumatoid synovium, gastric mucosa, skin and granulocytes. Each of these collagenases is also inhibited by purified preparations of α_2 -M but not by purified α_1 -AT (ref. 3).

Although the inhibitor has been characterised as a β_1 -serum protein with a molecular weight of approximately

40,000, the question of whether it represents a previously characterised serum protein, or a previously unrecognised protein of very low physiological concentration must await the results of purification of β_1 -AC which is in progress.

When whole serum was fractionated by gel filtration in Sephadex G-200 more than 90% of the collagenase-inhibitory capacity was demonstrable in those fractions containing α_2 -M. Although the inhibitory capacity of this antiproteinase greatly exceeds that of β_1 -AC in whole serum, this situation may well be reversed in certain tissue locations from which α_2 -M is excluded on account of its large molecular size, its prime physiological function possibly being to protect constituents within the circulatory system from proteolytic attack.

In a condition such as rheumatoid arthritis where there exists much accumulated evidence for an important role of neutral collagenase¹³ the operation of a natural inhibitor of the enzyme could be a factor in controlling the progress of the joint destruction. The finding of a small diffusible serum inhibitor, specific for collagenase, suggests that serum concentration of β_1 -AC might influence the rate of collagen catabolism in this condition. This hypothesis is being investigated.

The finding that four different human collagenases of similar activity when exposed to the β_1 -AC preparation showed different degrees of inhibition is of particular interest (Table 1). Although it is not yet possible to quantify this relationship on a molar basis the data suggest that each collagenase has a different susceptibility to the inhibitor, the granulocyte enzyme, for example, being far more resistant to inhibition than the gastric enzyme. Thus, alterations in the concentration of the serum inhibitor might be expected to have a differential effect on collagen breakdown in different tissues, and acquired or genetically determined inhibitor deficiencies could selectively "release" tissue col-

lagenases at different sites. Collagen catabolism at any one site would be the net result of the local concentration of collagenase, the local concentration of the specific inhibitor, and the susceptibility of the particular enzyme to β_1 -AC inhibition. Such ideas are, however, speculative at this stage.

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Are reptilian pulmonary receptors mechano- or chemosensitive?

BOTH birds and mammals possess pulmonary receptors which increase discharge in phase with each inspiration^{1–5}. In mammals the adequate stimulus of these receptors seems to be the total transpulmonary pressure which varies with both inflation volume and rate and sign of the volume change⁶. The lungs of birds, however, consist of a complex system of air tubes^{7,8} and unlike those of mammals are relatively inexpandable. Many avian pulmonary receptors have no mechanosensitivity, the adequate stimulus being the changes in airway CO₂ throughout the breathing cycle^{9–12}. So rate and degree of inflation of the respiratory system is signalled by different sensory modalities in birds and mammals. The demonstration that mammalian pulmonary receptors are partially inhibited by high alveolar

CO₂ (refs 13–15) and that some avian receptors may be mechanosensitive^{16,17} suggests that in the more phylogenetically ancient vertebrate classes it might be possible to locate a pulmonary receptor which has distinct mechano- and chemosensitive properties, thereby allowing speculation on the evolution of the avian and mammalian receptor types.

We report here the responses of turtle (*Chrysemys picta*) pulmonary receptors to both mechanical and chemical stimuli. This animal was chosen because reptiles represent the evolutionary stem group for both birds and mammals. Although many reptiles or even the more phylogenetically primitive amphibia might have served, our choice was governed by two facts. First, because of their diving habits, turtles experience alveolar CO₂ levels in the mammalian range¹⁸; second, in possessing a chambered, saccular lung that is constrained in its expansion by the exoskeleton¹⁹, turtles show a superficial resemblance to birds.

Turtles (600–1,200 g) were single-pithed and tidally ventilated with a constant volume, positive pressure, respiration pump. Single and multi-fibre nerve activity, in phase with artificial ventilation, was recorded in vagal slips using bipolar silver electrodes. Neural activity was amplified, visually displayed on an oscilloscope and audibly monitored. Tracheal air flow and intratracheal pressure (ITP), generated by the ventilator, were recorded with a pneumotachograph and pressure transducer respectively. All variables were stored on magnetic tape for later analysis on a Digital PDP Lab 8c mini-computer using conventional software.

Receptors with discharge modulated by artificial ventilation with air were of the slowly adapting low threshold type. Many units were active during the deflation phase when ITP approached that of the atmosphere (Fig. 1b), the average discharge frequency being 7.1 ± 1.4 impulses s⁻¹ at end deflation. Discharge increased with lung inflation and reached its maximum at peak ITP but none of the receptors seemed to respond to the rate of change in pressure either on inflation or deflation. Discharge frequency increased with inflation by 2.09 ± 0.32 impulses per s per cm H₂O increase in ITP, and discharge was maintained when artificial ventilation was held at either end inflation or end deflation. In response to maintained inflation, only 3 of the 14 single fibres investigated showed marked adaptation which was still less than 25% when calculated from index 1 of Davis *et al.*⁸. After removal of the sternum, 6 of the 14 units were located by punctate stimulation to the major septa which divide the lung into 8–10 chambers (Fig. 1a) and receptor discharge patterns were shown to be unaffected by pulmonary artery occlusion.

Addition of 10% CO₂ to the ventilating gas caused a rise

Fig. 1 *a*, Schematic diagram of the right lung of *Chrysemys picta* showing approximate site (●) of receptors located by punctate stimulation. *b*, Effect of 10% CO₂ on the discharge of a turtle's pulmonary receptors; continuous recording of intratracheal pressure (upper) and pulmonary receptor discharge (lower), 10% CO₂ introduced into the ventilating gas mixture at the arrow.

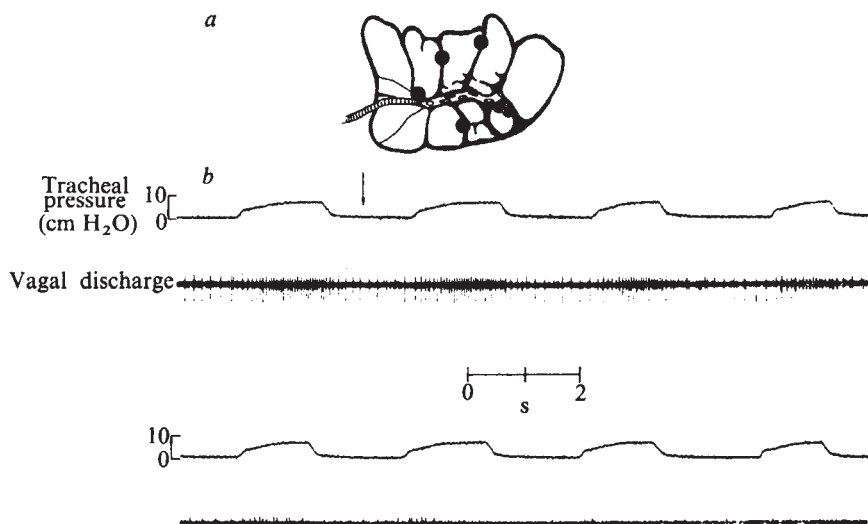


Table 1 Reduction in discharge rate of pulmonary receptors following the introduction of 10% CO₂ to the ventilating gas

Fibre no.	Peak inflation pressure (cm H ₂ O)	% Reduction in discharge rate associated with		
		End inflation	End deflation	One breath
1	—	33	100	66
2	5.5	46	100	65
3	7.0	27	60	26
4	4.5	63	100	68
5	6.5	100	100	100
6	6.5	100	100	100
7	9.0	37	7	27
8	9.0	56	73	52
9	9.0	53	80	45
10	7.5	36	—*	47
11	7.5	50	—*	38
12	5.0	33	30	44
13	5.0	55	60	62
14	8.0	100	—*	100

The difference between the discharge rates, when ventilating with air and 10% CO₂, is expressed as a percentage of the discharge rate with air.

*Discharge rates fell to zero while animals were breathing room air.

in threshold and a decrease in sensitivity of the receptors for any given pressure change compared with air inflation (Table 1). The increase in discharge rate on inflation was reduced to almost half (56%) the air inflation value and 3 units remained silent throughout inflation. For 5 units discharge ceased during lung deflation whereas, on the average, discharge rate during deflation was reduced by 74%. The decrease in activity began during the first inflation following a step change in CO₂ content of the ventilating gas and continued to fall over several cycles of ventilation (Fig. 1b) (using tidal ventilation, 2–3 breaths were required before the expired gas composition equalled that of the inspired gas). High CO₂ did not seem to affect the adaptation rate to maintained inflation. The effects of ventilating with 5% CO₂ in air were about half as pronounced as the effects of 10% CO₂. Since some units were completely silenced by ventilation with air containing 10% CO₂, any changes in lung compliance induced by the presence of CO₂ cannot explain this reduction in receptor discharge. Lung compliance, however, was decreased in four turtles whereas three showed no change in compliance when 10% CO₂ was added to the ventilating gas. In only one case did the fall in compliance (35%) match the fall in the maximum discharge frequency, at peak ITP, of the receptor being recorded.

This work has shown that although all pulmonary receptors we have monitored are mechanosensitive, some are extremely sensitive to CO₂, being completely inhibited by pulmonary concentrations of 10%. This sensitivity to CO₂ resembles that shown by avian pulmonary chemoreceptors. On the other hand, those receptors exhibiting only slight sensitivity to CO₂ have responses similar to those of mammalian pulmonary mechanoreceptors. For these reasons, the reptilian receptor type may be viewed as the functional precursor of those in higher vertebrates. It also suggests that the reflex respiratory responses to intrapulmonary CO₂ shown by reptiles²⁰, birds²¹ and mammals²² are phylogenetically ancient.

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Muscle activity decreases rate of degradation of α -bungarotoxin bound to extrajunctional acetylcholine receptors

DURING development of skeletal muscle, and after denervation or reinnervation of adult muscle, there are significant changes in the number of extrajunctional acetylcholine (ACh) receptors, measured both by sensitivity to ACh and by binding of α -¹²⁵I-bungarotoxin (for review, see ref. 1). Muscle activity has been shown to be a major factor in controlling the level of extrajunctional receptors. Direct electrical stimulation of denervated muscle both *in vivo* and *in vitro* decreases the sensitivity of the extrajunctional membrane to ACh^{2–5}, whereas chronic postsynaptic blockade of innervated muscle *in vivo* increases its sensitivity^{6,7}. Muscle activity could alter the level of extrajunctional receptor by changing rates of synthesis or degradation of the receptor.

We have investigated the effect of direct electrical stimulation in organ culture on the number of extrajunctional ACh receptors in denervated muscles and on the degradation of α -¹²⁵I-bungarotoxin bound to extrajunctional receptors. We have found that the rate of toxin breakdown is slowed in conditions of stimulation which over several days reduce the number of receptors. This result suggests that activity decreases receptor levels by decreasing receptor synthesis, rather than by affecting receptor degradation.

Attempts have been made^{8–11} to measure degradation of ACh receptors indirectly by binding α -¹²⁵I-bungarotoxin to them and monitoring the loss of radioactivity from the tissue. The loss of radioactive toxin from extrajunctional receptors in cultured rat diaphragm muscle involves degradation of toxin with release of ¹²⁵I-monoiodotyrosine into the medium and has many of the characteristics of intracellular protein breakdown⁹. Toxin bound to the ACh receptors of myotubes in primary cell culture is degraded by a similar process, and experiments by Devreotes and Fambrough have provided strong indirect evidence that in myotubes the rate of toxin loss reflects the normal rate of receptor turnover¹¹. Although receptor breakdown has not yet been measured directly, the available evidence suggests that breakdown of receptors can be followed by measuring degradation of bound toxin.

In the present investigation, left hemidiaphragms from male white rats (50–60 g) were surgically denervated, and 5 d later, left and right hemidiaphragms were pinned to separate culture dishes coated with Sylgard, as described previously⁹. Dishes for stimulation contained platinum electrodes embedded in recessed troughs. Muscles were mounted over, but not in contact with, the electrodes and were stimulated continuously by suprathreshold pulses of

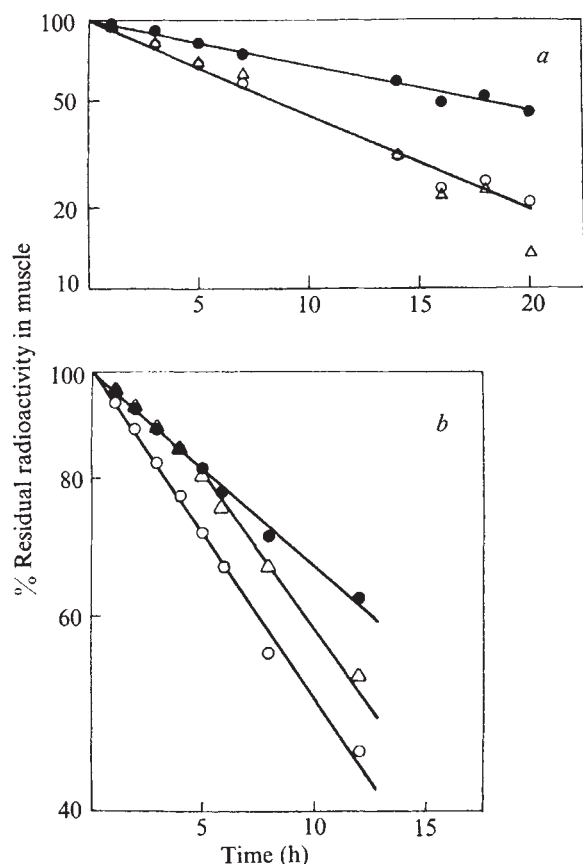


Fig. 1 Effect of muscle activity on degradation of α - ^{125}I -bungarotoxin bound to extrajunctional ACh receptors in denervated muscle. Hemidiaphragms from animals that had been denervated 5 d previously were labelled *in vivo* by injection of radioactive toxin and transferred 3 h later to organ culture. After several changes of medium to reduce the level of unbound toxin in the muscle, release of radioactivity from each muscle was monitored by sampling the medium, and radioactivity remaining in each muscle at the end of the experiment was measured. Each value for the left hemidiaphragm was corrected by subtracting, on a per weight basis, the corresponding value for the right hemidiaphragm from the same animal. The total amount of radioactivity initially associated with extrajunctional receptors was calculated as the sum of the corrected values of radioactivity released into the medium plus that remaining in the muscle. Stimulation was at 2 Hz. *a*, One muscle ($\bullet$) was stimulated in normal culture medium, one (Δ) was stimulated in culture medium containing 10^{-5} g ml $^{-1}$ tetrodotoxin, and the other ($\circ$) was not stimulated. Duplicate 0.1-ml samples of medium were taken for each time point. *b*, All muscles were incubated for 16 h, and release was monitored during the last 12 h of the experiment. One muscle ($\bullet$) was stimulated continuously, one (Δ) was stimulated only during the first 6 h of incubation, and the other ($\circ$) was not stimulated. Duplicate 0.5-ml samples of medium were taken for each time point.

2-ms duration and alternating polarity. In experiments testing the effects of electrical stimulation on ACh sensitivity, muscles were stimulated at a frequency of 10 Hz, comparable to the discharge frequency of single units in the phrenic nerve⁵. Muscles were removed from the incubator at the end of the culture period, and the effectiveness of stimulation through the electrodes in the dish was determined by monitoring the production of action potentials in different areas of the muscle. In general about 15 out of 20 fibres sampled gave action potentials in response to stimulation, suggesting that a high proportion of fibres had been active in culture.

Direct electrical stimulation reduced the extrajunctional sensitivity developed by denervated muscles in culture. Fibres in muscles placed in culture 5 d after denervation and cultured for 4 d with continuous electrical stimulation

showed lower extrajunctional ACh sensitivities than fibres in muscles similarly treated but not stimulated (Table 1). Muscle fibres denervated at the time of culture and continuously stimulated in culture for 4 d also showed lower sensitivities than those from unstimulated muscles (Table 1). These results are similar to those of Purves and Sakmann⁵, although the effects that we observe are not as large. In their experiments, stimulation was carried out for 7–8 d, and only fibres that were judged to have been continuously active were examined.

Stimulation of muscles for only 24 h in these same conditions produced no detectable change in extrajunctional sensitivity. The extrajunctional sensitivity of 5-d denervated muscles stimulated in culture for 24 h was 103 ± 10 mV nC $^{-1}$ (mean $\pm$ s.e., $n=20$), whereas that of unstimulated muscles was 100 ± 6 mV nC $^{-1}$ ($n=20$). In slightly different conditions of culture, Purves and Sakmann⁵ reported a small decrease in extrajunctional sensitivity in muscles stimulated for 24 h.

To determine whether an increased rate of receptor degradation contributes to the observed reduction of extrajunctional sensitivity the loss of α - ^{125}I -bungarotoxin bound to extrajunctional receptors was examined. Radioactive toxin was injected into the thorax of denervated rats, and 3 h later the left and right hemidiaphragms were removed and cultured as described above. The time course of loss of bound ^{125}I -toxin was followed by monitoring the appearance of radioactivity in the medium and measuring the radioactivity in the muscle at the end of the experiment. Values from right hemidiaphragms which were innervated at the time of culture were used to correct for the contribution of toxin bound to sites other than extrajunctional receptors⁹.

The time course of degradation of α - ^{125}I -bungarotoxin was studied in unstimulated muscles and in muscles stimulated at 2 or 10 Hz (Fig. 1). In all experiments, toxin degradation was slower in the stimulated muscles. The release of radioactivity could be described as a single exponential process whose half time ranged in unstimulated muscles from 8 to 14 h and in stimulated muscles from 16 to >30 h. When tetrodotoxin (10^{-5} g ml $^{-1}$) was included in the culture medium, the stimulating current did not elicit muscle activity, and the time course of loss of radioactivity from these muscles was indistinguishable from that of the unstimulated muscles (Fig. 1*a*). Thus the slower loss of radioactivity from stimulated muscle is related to activity of the fibres.

The onset of the effect of activity and its reversibility were determined in muscles stimulated during only part of the incubation. When stimulation was terminated, the rate of toxin degradation recovered to the control value within several hours (Fig. 1*b*). Conversely, a decreased rate of degradation was observed within a few hours after stimulation was begun (not shown).

Table 1 Effect of stimulation on extrajunctional ACh sensitivity of muscles in culture

	ACh sensitivity (mV nC $^{-1}$)	
	Unstimulated	Stimulated
<i>a</i> , Denervated 5 d, cultured 4 d	322 ± 35 (24)	50 ± 9 (19)
<i>b</i> , Cultured 4 d	83 ± 8 (56)	18 ± 2 (57)

In one series of experiments denervated muscles were cultured, and either stimulated continuously at 10 Hz or not stimulated (*a*). In another series normal muscles were treated similarly (*b*). After the period in culture, extrajunctional sensitivity was determined by iontophoretic application of ACh from micropipettes that contained 2 M acetylcholine bromide and had resistances of 80–120 M Ω . No attempt was made to select fibres that had been active in culture. Values reported are mean $\pm$ s.e.m. for three pairs of denervated muscles and for eight pairs of normal muscles. In each series, the stimulated muscles showed lower sensitivity ($P < 0.001$).

The radioactivity remaining in the extrajunctional regions of stimulated muscle at the end of the experiment was extracted with detergent and examined by zone sedimentation. The sedimentation profile had a single major peak corresponding to toxin-receptor complex, and except for the larger amount of radioactivity did not differ appreciably from that obtained with extracts of unstimulated muscle. Therefore, the radioactive toxin in stimulated muscle remains bound to receptor, suggesting that stimulation may slow the process of toxin degradation at an early step.

If degradation of toxin is an adequate measure of normal receptor breakdown, the effect of activity on the rate of receptor degradation that we have observed is in the wrong direction to account for its effect on extrajunctional receptor levels. One interpretation of this result is that the effect of muscle activity on degradation may not be a specific one—since activity has been shown to decrease the average rate of protein degradation¹⁴—but that receptor levels may be regulated by affecting rates of synthesis. Experiments showing that denervated, but not innervated, muscles accumulate newly synthesised receptor are consistent with this idea^{21,13}.

We conclude that muscle activity prolongs the half life of α -¹²⁵I-bungarotoxin bound to extrajunctional ACh receptors in denervated rat diaphragm. This effect is reversible and occurs at low frequencies of stimulation. These findings suggest that muscle activity may slow the normal degradation of the extrajunctional ACh receptor, and they provide indirect evidence for an effect of activity on the synthesis of the ACh receptor.

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Lanthanum inhibits brain adenylate cyclase and blocks noradrenergic depression of Purkinje cell discharge independent of calcium

It has been reported that lanthanum (La^{3+}) can reversibly antagonise the depressant effect of noradrenaline (NA) on cerebral cortical neurones^{1,2}. Because this ion is known to interfere with membrane actions of calcium^{3–7}, it was proposed, on the basis of this and other data, that the inhibitory effects of NA are mediated through a mechanism involving transmembrane movement of Ca^{2+} (refs 1 and 2). On the other hand, evidence from other laboratories has

suggested that the effects of NA are primarily related to the activation of a NA-sensitive adenylate cyclase, with a consequent increase in the intracellular concentration of cyclic AMP^{8–10}. We now report that La^{3+} is a potent inhibitor of brain adenylate cyclase and suggest that this ion blocks responses of cerebellar Purkinje cells to NA by means of a mechanism involving the inhibition of NA-stimulated increases in cyclic AMP, independent of an effect on calcium.

Figure 1 (bottom curve) shows the effect of lanthanum chloride on the rate of formation of cyclic AMP from ATP in an homogenate of rat cerebellum. Enzyme activity was inhibited by concentrations of La^{3+} as low as $0.1 \mu\text{M}$: 50% inhibition occurred at $2 \mu\text{M}$ and almost complete inhibition at $100 \mu\text{M}$. In addition to inhibiting basal adenylate cyclase activity, La^{3+} also blocked enzyme stimulation by NA (Fig. 2b). In the conditions used for assaying adenylate cyclase (in which theophylline was present), phosphodiesterase (PDE) activity was largely inhibited. The little PDE activity that remained was unaffected by La^{3+} , indicating that the observed decrease in cyclic AMP formation was due to inhibition of adenylate cyclase and not to stimulation of PDE. When theophylline was omitted from the reaction medium, La^{3+} caused a small stimulation of PDE activity

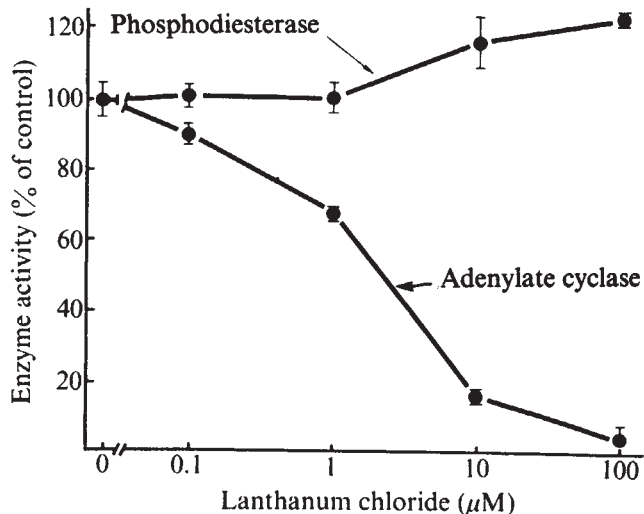


Fig. 1 Effect of lanthanum chloride on adenylate cyclase ($I_{50} = 2 \mu\text{M}$, lower curve) activity and PDE (upper curve) activity in a homogenate of rat cerebellar cortex. The values shown are the mean ($\pm$ mean deviation) of triplicate determinations. Control activities were 241 ± 4 pmol per mg protein per min for adenylate cyclase and 16.4 ± 0.8 pmol per mg protein per min for PDE. To measure adenylate cyclase activity, 1-mm slices of cerebellar cortex were homogenised (15 mg ml^{-1}) in 6 mM Tris-maleate buffer, pH 7.4. Cyclic AMP formation in these homogenates (or in particulate fractions prepared from the washed 30,000g pellet of the homogenate) was measured in an assay system (0.3 ml) containing 80 mM Tris-maleate, pH 7.4, 10 mM theophylline, 6 mM MgSO_4 , 1.5 mM ATP and tissue (1 mg wet weight), with or without lanthanum chloride. The reaction (3 min at 30°C) was initiated by addition of ATP, terminated by boiling for 2 min, and then centrifuged at low speed to remove insoluble material. Cyclic AMP in the supernatant was measured by the method of Brown *et al.*²⁰ with appropriate blanks. In some cases, samples were partially purified by ion-exchange chromatography to remove metal ions and ATP before measurement of cyclic AMP by the protein binding assay. In the experimental conditions used, enzyme activity was linear with respect to time and enzyme concentration. At concentrations less than $250 \mu\text{M}$, La^{3+} had no effect on non-enzymatic conversion of ATP to cyclic AMP. For measurement of PDE activity, assay conditions were identical to those described above except that $0.1 \mu\text{M}$ (approximately 30 nCi) ^3H -cyclic AMP (New England Nuclear) was used in place of ATP, theophylline was omitted, and incubation time was 1.5 min. PDE activity was measured as the rate of formation of tritiated adenosine³⁰.

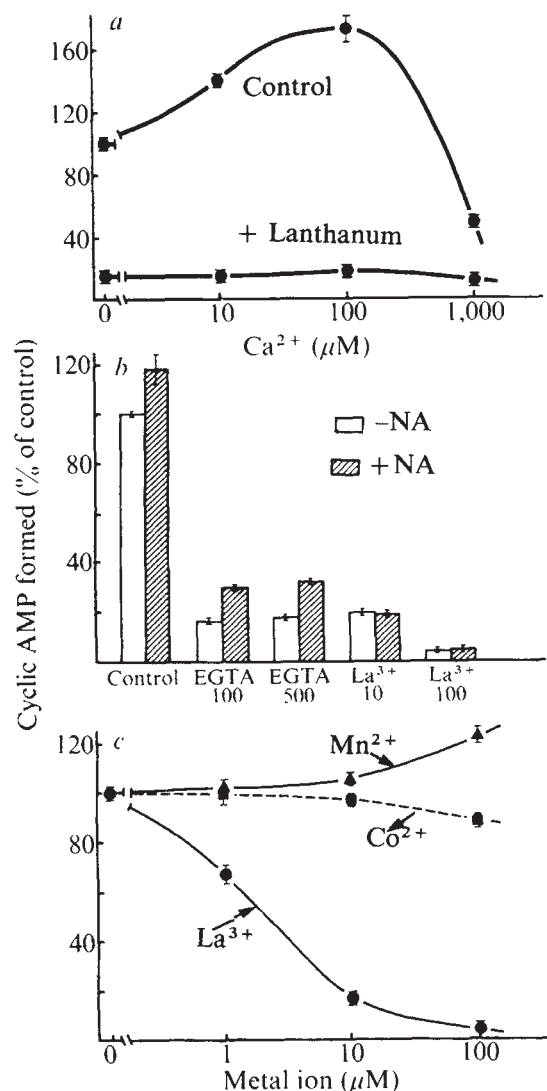


Fig. 2 Differential effects of La^{3+} and calcium on adenylate cyclase activity. *a*, Addition of exogenous calcium chloride (up to 1,000 μM) failed to reverse the inhibition of enzyme activity by 10 μM La^{3+} . Upper curve, control; lower curve, +10 μM La^{3+} . *b*, Chelation of endogenous calcium by 100 or 500 μM EGTA selectively reduced basal enzyme activity but did not affect stimulation by NA, whereas 10 or 100 μM La^{3+} inhibited basal and abolished hormone-stimulated activities. Open columns, no NA; hatched columns, +250 μM NA. *c*, Cobalt ($\blacksquare$) and manganese ($\blacktriangle$), which also interfere with membrane effects of calcium, had little inhibitory effect on adenylate cyclase. The values shown in the figure are the mean ($\pm$ mean deviation) of two replicate samples, each assayed for cyclic AMP content in duplicate. $\bullet$, La^{3+} . Control activity in (*a*) and (*c*) was 167 ± 3 pmol per mg protein per min and in (*b*) it was 171 ± 2 pmol per mg protein per min.

(Fig. 1, top curve). Results similar to those in Fig. 1 were also seen when washed particulate fractions, instead of homogenates, were used as a source of enzyme. In other experiments we have observed that the lanthanide series element, neodymium, also inhibits adenylate cyclase.

Low concentrations of calcium stimulate basal adenylate cyclase activity of brain homogenates, whereas higher concentrations are inhibitory^{11,12}. Also, in some non-neural tissues, certain hormone-stimulated increases in cyclic AMP

depend on calcium¹³. In the experiments just described, inhibition of adenylate cyclase by La^{3+} might have been due to displacement of small amounts of endogenously bound calcium from the enzyme, preventing the usual stimulatory effect of this ion and causing an apparent inhibition of activity. For several reasons, however, we feel that such a mechanism is unlikely. First, the addition of a 100-fold excess of calcium over La^{3+} did not reverse the inhibition of adenylate cyclase (Fig. 2*a*), even though high concentrations of calcium are known to overcome partially the inhibitory effects of La^{3+} on calcium-dependent processes⁶. Second, chelation of endogenous calcium by EGTA reduced adenylate cyclase activity but did not eliminate it, whereas La^{3+} almost certainly abolished enzyme activity (Fig. 2*b*). Third, the action of La^{3+} differed qualitatively from the effect of calcium chelation, since La^{3+} reduced basal activity and totally eliminated NA-stimulated activity, whereas EGTA selectively blocked only basal activity (Fig. 2*b*). Finally, cobalt and manganese, which are also known to inhibit membrane effects of calcium^{14,15}, had almost no inhibitory effect on cerebellar adenylate cyclase activity at concentrations as great as 100 μM , the largest dose tested (Fig. 2*c*). Manganese actually stimulated enzyme activity to a small degree.

In electrophysiological studies we found, as observed previously¹⁰, that most cerebellar Purkinje neurones respond to the iontophoretic application of NA with a decrease in spontaneous firing rate. Figure 3 shows that La^{3+} , applied iontophoretically during a series of NA pulses, reversibly antagonised this inhibition. In contrast, La^{3+} had no effect on the inhibition of Purkinje cell firing due to the iontophoretic application of γ -aminobutyric acid (GABA), a transmitter which does not stimulate cyclic AMP synthesis in the cerebellum¹⁶. La^{3+} caused strong antagonism of NA responses in nine of 11 neurones examined while not affecting responses to GABA in any of seven cells. In other preliminary experiments, La^{3+} failed to block the inhibitory actions of iontophoretically-applied cyclic AMP in three of four cells examined.

Cyclic AMP and its derivatives mimic the depressant effects of NA on most cerebellar Purkinje cells^{9,17,18}. Our results demonstrate that La^{3+} , a potent inhibitor of cyclic AMP synthesis in the cerebellum, also blocks the inhibitory effects of NA on Purkinje cell discharge. Other studies have shown that exogenous La^{3+} binds to cell membranes^{19,20} and therefore, *in vivo*, would have access to the regulatory subunit of NA-sensitive adenylate cyclase, also located on the plasma membrane⁸. Because stimulation of this enzyme seems to result in an accumulation of cyclic AMP in Purkinje cells²¹, we propose that the NA-blocking action of La^{3+} *in vivo* is a result of direct inhibition by this ion of adenylate cyclase activity. It seems unlikely that La^{3+} acts presynaptically, since the inhibitory effects of NA seem to be the result of a direct postsynaptic action²²⁻²⁵.

We have shown that certain other heavy metals, such as lead, can also inhibit brain adenylate cyclase activity, perhaps by interacting with functional sulphhydryl groups on the enzyme molecule^{26,27}. Our preliminary experiments indicate that lead, too, can block the depressant effects of NA on Purkinje cell discharge. In contrast, the non-heavy metals cobalt and manganese, which at similar concentrations have little effect on adenylate cyclase activity in homogenates, also fail to block the effects of iontophoretically applied NA in Purkinje and cerebral cortical neurones²⁸. (In contrast to our results with the cerebellum, Yarborough *et al.*^{1,2} have reported that cobalt and manganese block NA responses of cerebral cortical neurones. Possible reasons for this discrepancy have been discussed²⁸.) Because cobalt and manganese, like La^{3+} , are known to interfere with the effects of calcium^{14,15}, calcium antagonism *per se* is not sufficient to block the NA responses of cerebellar Purkinje cells. This conclusion is supported by the observed

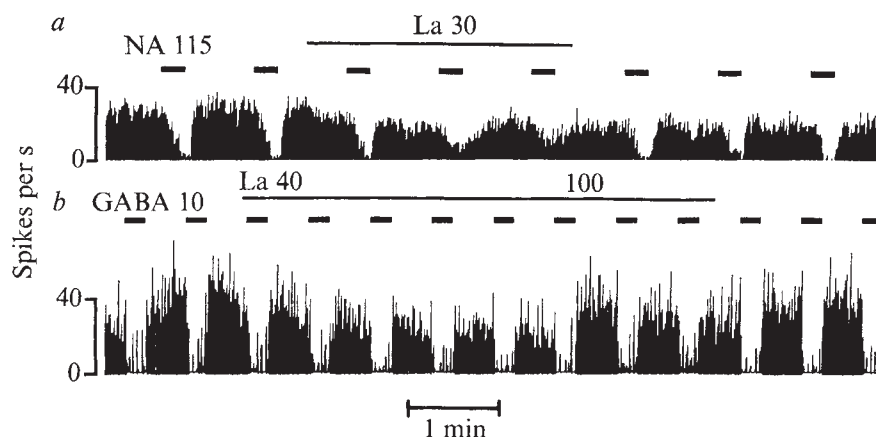


Fig. 3 Ratemeter records of Purkinje neurone discharge. *a*, Antagonism of NA-induced inhibitions by iontophoresis of La^{3+} . *b*, GABA-induced inhibitions (in a different animal) were unaffected by La^{3+} . The numbers after each drug represent the ejection current in nanoamperes, and the underlying bar is the duration of ejection. For these studies, 11 male Sprague-Dawley rats (150–200 g) were anaesthetised with 1% halothane and allowed to breathe spontaneously. Recordings were made from Purkinje cells in the vermis, lobules VI and VII, identified by anatomical location and their characteristic discharge of complex and single spikes³¹. Five-barrelled micropipettes with 4–8- μm tips were used to record extracellular action potentials of spontaneously active single Purkinje neurones and to apply substances at the site of recording by micro-iontophoresis. The centre recording barrel was filled with 5 M NaCl; three side barrels were filled with drugs by centrifugation. The remaining side barrel, filled with 3 M NaCl, was used to balance drug ejection and holding

currents by automatically passing an equal and opposite charge. Drug solutions were: 0.5 M l-NA HCl, pH 4.5; 1.0 M GABA, pH 40; 0.25 M cyclic AMP, pH 7.5; 0.2 M LaCl , pH 7.4 in 0.1 M NaCl. A crystal clock controlled the iontophoresis circuit³², so that pulses of uniform current and duration could be applied at equal intervals²⁸. Iontophoretic artefacts of current, drug pH, and local anaesthesia were controlled carefully, as before¹⁸. For each cell studied, La^{3+} was tested at least twice against agonist responses. Data from cells which did not completely recover the agonist response after cessation of La^{3+} application were discarded²⁸.

failure of exogenous calcium to overcome La^{3+} inhibition of adenylate cyclase *in vitro* and by the qualitatively and quantitatively different effects of La^{3+} and EGTA. Thus although the results presented here do not diminish the role of La^{3+} as a calcium antagonist or preclude some indirect interaction of La^{3+} and calcium on cyclic AMP metabolism in the intact Purkinje cell, they indicate that La^{3+} has potent effects on other biochemical mechanisms which may be involved in synaptic transmission.

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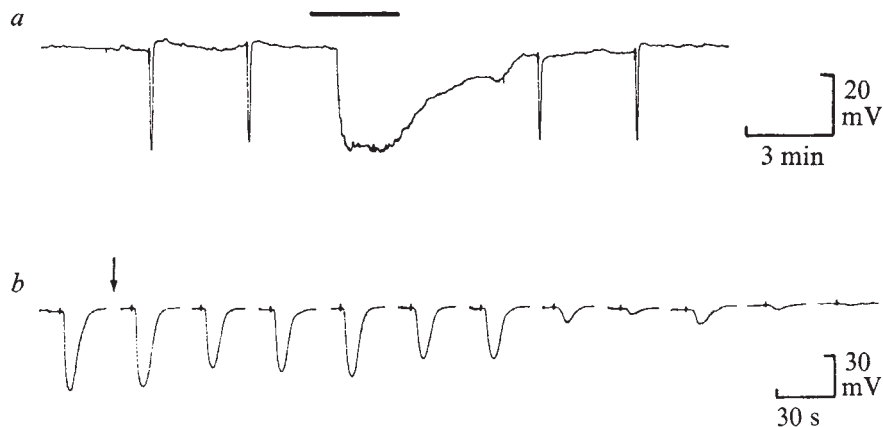
Actions of a dopamine analogue and a neuroleptic at a neuroglandular synapse

SUBSTANCES which mimic or inhibit the actions of dopamine in the vertebrate central nervous system (CNS) have become of increasing interest since the discovery of their potential clinical importance¹. It has been suggested that the receptors involved are closely associated with an adenylate cyclase responsible for the formation of cyclic AMP by homogenates of the neostriatum and limbic regions of the brain in the presence of dopamine². In favour of this idea, substances believed to be agonists for central dopamine receptors also stimulate the production of cyclic AMP in such homogenates^{2,3}, and neuroleptics, which are believed to act by blocking dopaminergic transmission, inhibit the action of dopamine on homogenates^{2,4}. It is difficult, however, to obtain information about the physiological mode of action of these compounds at the cellular level in the CNS. We report here the effects of a dopamine-like agonist and a neuroleptic on an alternative system which enables investigation of intracellular responses. Our results are consistent with the idea that both substances act directly on postsynaptic dopamine receptors.

The substances tested were 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (ADTN) which, as predicted by Woodruff⁵, has been found to be an analogue of dopamine both physiologically⁶ and biochemically⁷; and flupenthixol, of which the *cis* isomer (α -flupenthixol) is a potent neuroleptic⁷ and inhibitor of the stimulation of cyclic AMP formation by dopamine, whereas the *trans* isomer (β -flupenthixol) is inactive⁴. The preparation used consisted of the innervated cockroach salivary gland which contains acinar cells responsive to dopamine and for which there is evidence of dopaminergic transmission^{8,9}. Both externally applied dopamine and nerve stimulation produce hyperpolarisations.

Responses to nerve stimulation and to ADTN are shown in Fig. 1a. A substantial hyperpolarisation was obtained on application of less than 1 μM ADTN. Figure 1b illustrates the progressive inhibition by 0.5 μM α -

Fig. 1 *a* and *b*, Intracellular records from two cockroach salivary gland cells. The unlabelled downward deflections are hyperpolarisations in response to short trains of stimuli delivered at about 3-min intervals to the salivary duct nerve at a frequency of 100 Hz. *a*, Bar indicates the period during which $0.75 \mu\text{M}$ ADTN was present in the bathing solution flowing through the bath; *b*, $0.5 \mu\text{M}$ α -flupenthixol was added after the arrow; the block developed progressively. The preparation is described in ref. 11.



flupenthixol of successive responses to nerve stimulation. In Fig. 2 the action of α -flupenthixol is shown both on responses to applied dopamine and on approximately matching responses to nerve stimulation. The dopamine responses were reduced to a somewhat greater extent; this does not support the view that the effect of α -flupenthixol is predominantly presynaptic¹⁰.

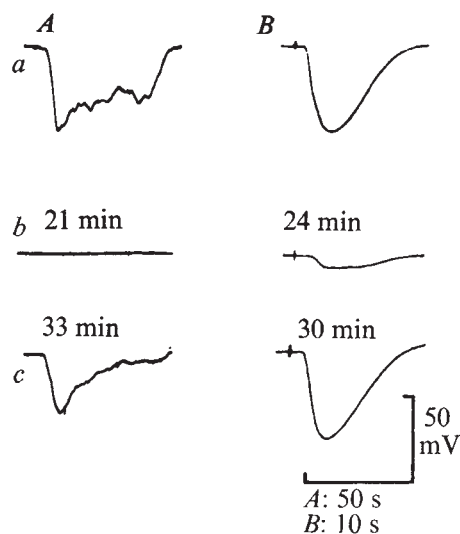


Fig. 2 Intracellular records from a cockroach salivary gland cell. Downward deflections are hyperpolarisations. Responses to dopamine (*A*) and to trains of nerve stimuli (*B*) before (*a*) and in the presence of (*b* and *c*) $0.5 \mu\text{M}$ α -flupenthixol. *a* and *b*, Concentration of dopamine was $0.18 \mu\text{M}$ and the train consisted of 4 stimuli at 100 Hz. *c*, Concentration of dopamine was raised to $2 \mu\text{M}$ and the number of stimuli to 10; the responses show that the blockade is at least partly surmountable. *b* and *c*, Time for which the α -flupenthixol had been present is shown in each record.

Our results show that at this synapse ADTN is a potent agonist and α -flupenthixol a potent antagonist: they thus reinforce the analogy between the electrophysiological effects of dopamine and its ability to stimulate cyclic AMP production.

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Plasma noradrenaline increases with age

WE measured plasma noradrenaline (NA) levels in about 20 individuals who were to serve as normal control subjects and noted that older subjects tended to have higher NA levels. Extending the study to teenage and elderly subjects revealed that basal levels of plasma NA correlate with age and that the increase in plasma NA in response to stress is similarly related to age. There is considerable evidence that sensitivity to NA and NA metabolism change with increasing age. In rabbits and cats the threshold for cardiovascular response to low levels of NA decreases with old age¹. In ageing rats uptake of NA into the heart is greater than in young animals² and there is a diminished inotropic response of aged rat myocardium to a fixed concentration of NA (ref. 3). Cardiac monoamine oxidase activity increases severalfold during the life span of a rat while dopa decarboxylase decreases during the first year². In man, propranolol, which blocks β -adrenergic receptors, reduces heart rate and cardiac output during exercise, but this effect is considerably smaller in older subjects⁴. The response of heart rate to hypoxia and hypercapnia is attenuated in older men⁵.

The enzyme that synthesises NA, dopamine- β -hydroxylase, is secreted along with NA during sympathetic nerve activity⁶. Blood levels of dopamine- β -hydroxylase increase with age⁷. Vanillylmandelic acid, a major metabolite of NA, is excreted in increasing amounts through childhood and adolescence⁸.

The possible relationship of blood levels of NA to age is important in clinical studies; several investigators have reported elevated plasma levels of NA in essential hypertension^{9–16}, but the ages of the patients with hypertension may have been different from the control subjects.

We measured NA in normotensive subjects, in good health, ranging from 10 to 65 years of age. None was taking medication and all were asked to abstain from coffee, tea, cola, cocoa and cigarettes before testing but otherwise followed their normal diet and activity. The testing procedure and its purpose were thoroughly explained to all subjects and written consent obtained. They were asked to lie supine and the needle of a "heparin lock" was placed in an antecubital vein of their non-dominant arm and after

at least 20 min of rest a 12-ml blood sample was taken. We had previously determined that noradrenaline levels are consistently higher immediately following venepuncture than after a 20-min rest, but do not decrease further for 3 h. Subjects were then asked to stand and a blood sample obtained after 5 and 10 min. Most subjects were then asked to squeeze a hand dynamometer as hard as possible (Jamar Dynamometer, Asimow) and to maintain 30% of the maximum force for 5 min (ref. 17) at which time a final blood sample was obtained. Pulse and blood pressure were measured while subjects were lying and standing and the pulse rate was measured during the last minute of exercise. Blood samples were mixed with ACD anticoagulant, stored on ice and centrifuged within 30 min, the plasma separated and stored at -100°C until assayed. NA was measured by a modification of the method of Henry *et al.*¹⁸ which uses the conversion of NA to ^3H -adrenaline by phenylethanolamine-*N*-methyl-transferase catalysed transfer of a ^3H -methyl group from ^3H -S-adenosyl-methionine. The assay could detect 20 pg ml^{-1} of NA.

The mean resting blood pressure of these subjects was 115/70 and after standing 5 min this changed to 112/75 and after 10 min was 112/78. The mean heart rate of these subjects increased from a resting level of 73 beats min^{-1} to 89 after they stood for 5 min and 87 after standing for 10 min. Five minutes of isometric exercise increased their heart rate to $106\text{ beats min}^{-1}$. Normotension was a criterion for entry into this study and in these subjects resting systolic blood pressure ranged from 95 to 135 and diastolic pressure from 50 to 85; neither were related to age. There was, however, a significant correlation between plasma NA levels and age (Fig. 1). This correlation was apparent whether the subjects were recumbent, standing for 5 or 10

min or performing isometric exercise. The correlation of NA with age was about $r=0.5$ for all postures and exercise. Erect posture and exercise evoked a greater increase in plasma NA levels in older subjects; thus the slope of the regression lines in Fig. 1 are steeper for those manoeuvres resulting in the highest NA levels. During exercise there was an increase in heart rate by $34 (\pm 3)\text{ beats min}^{-1}$ (s.e.m.), but there was no significant correlation of this increase with age ($r=0.04$). In older individuals, however, there was a greater increase in NA levels during exercise and the increase in NA levels between rest and exercise significantly correlates with age ($r=0.48$, $P<0.01$). Older subjects have higher basal levels of plasma NA and those subjects with high basal NA levels have a larger increase in their NA levels after exercise. Thus the absolute increase in NA levels after exercise correlates with age but the NA increase expressed as a percentage of the resting level does not ($r=0.01$). Although plasma NA levels after exercise are quite variable (coefficient of variance=101%), when expressed as a percentage increase over the resting NA level the increments are less varied (coefficient of variance=58%) because those individuals with the highest basal NA levels tend to have the largest NA increases.

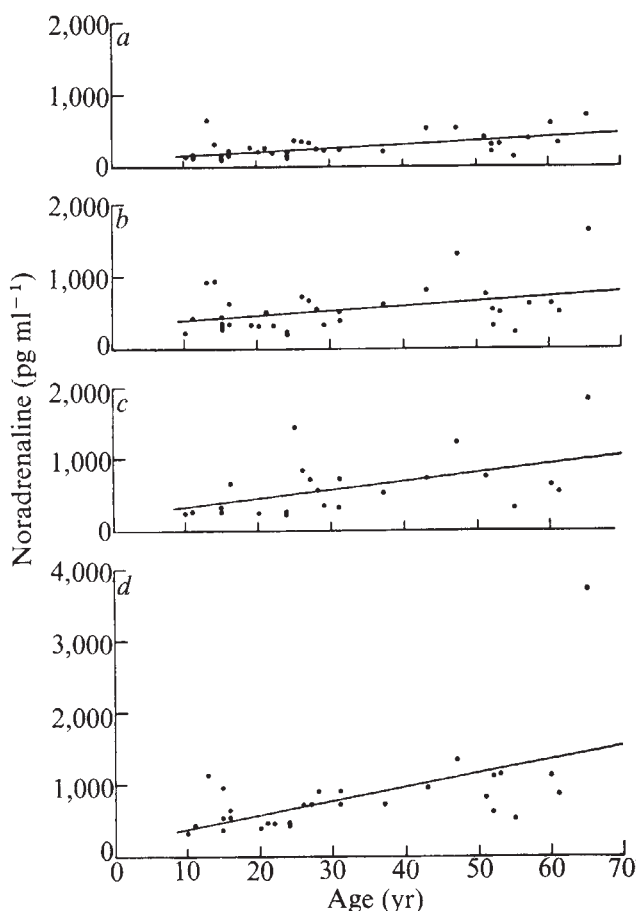
Of the 38 individuals in this study, seven complained of feeling faint or dizzy while standing. Some had a frank vasovagal reaction with yawning, bradycardia and pallor and could not continue the test procedure. These subjects were all under the age of 25, and had basal NA levels ranging from 130 to 650 pg ml^{-1} ; when correlations of NA with age are made without these individuals the correlation coefficients are at least as high as when they are included.

The 16 males in this study were aged from 10 to 65 and had a mean age of $30.3 \pm 4.0\text{ yr}$, and a mean resting plasma NA level of $270 \pm 42\text{ pg ml}^{-1}$ ($\pm$ s.e.m.). The 22 females were aged from 11 to 61 and had a mean age of $30.6 \pm 3.7\text{ yr}$, and NA of $307 \pm 31\text{ pg ml}^{-1}$ ($\pm$ s.e.m.). The difference in plasma NA levels between males and females is not significant.

Plasma levels of NA gradually increase with age in resting subjects and older subjects have a greater increase in plasma NA levels in response to a uniform stress. These observations may be attributed to increased secretion of NA or to decreased catabolism or reuptake of the secreted NA in older subjects. Increased secretion of NA should lead to increased excretion of urinary metabolites of NA, and vanillylmandelic acid is excreted in increasing amounts from birth to age 20 (ref. 8). Rat myocardium becomes less responsive to the effects of NA with increasing age because of NA receptor insensitivity³. Decreased responsiveness to the effects of NA in man might lead to a compensatory increased NA secretion during rest and in response to stress. Older subjects have a diminished response to β blockade with propranolol⁴ and this may be a reflection of decreased adrenergic receptor sensitivity with age. If the sensitivity of adrenergic receptors does diminish with age, then the response not only to propranolol but to many other noradrenergic agonist and antagonist drugs may diminish with increasing age.

Although the incidence of hypertension increases with age, in most reports of elevated plasma levels of NA in hypertension the control subjects have not been age-matched. These observations suggest that in studies of plasma levels of NA or of responsivity of the sympathetic nervous system to stress and drugs, age should be considered in choosing appropriate control subjects.

Fig. 1 Plasma noradrenaline in pg ml^{-1} of subjects aged 10 to 65 yr while supine and resting (a), standing for 5 (b) or 10 (c) min or performing isometric exercise (d).



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Depressed cardiac cyclic GMP-dependent protein kinase in spontaneously hypertensive rats and its further depression by guanethidine

BIOCHEMICAL modifications in cardiac hypertrophy, secondary to high blood pressure, in spontaneously hypertensive (SH) rats include reduced receptor reactivities to isoproterenol^{1,2} and glucagon³, and lower contents of cyclic AMP and cyclic GMP due to aberrations in adenylate and guanylate cyclases or phosphodiesterases, or both. Also possibly affected are cyclic AMP-dependent protein kinase (A-PK) and cyclic GMP-dependent protein kinase (G-PK), enzymes that presumably mediate the effects of the respective cyclic nucleotide *in vivo*. An accurate assay for G-PK from mammalian tissues is possible in the presence of stimulatory modulator (specifically stimulating G-PK), either as a separated and purified form⁴ or as a mixture with inhibitory modulator (specifically inhibiting A-PK) in a form of crude protein kinase modulator^{5,6}. We have examined possible involvements of cardiac G-PK, relative to A-PK, in chronic hypertension and possible effects of guanethidine, a hypotensive drug, on the two key enzymes. We found that the

cardiac level of G-PK in spontaneously hypertensive rats was lower than that in normotensive rats, whereas that of A-PK was the same in both groups. Guanethidine depressed the former enzyme further without affecting the latter.

Breeding pairs of SH (SH/N) and normotensive (WKY/N) rats were obtained from the NIH. WKY/N are descendants of the original Kyoto Wistar colony from which the SH mutant was bred by Okamoto⁷.

There was less protein kinase activated by cyclic GMP in extracts of heart ventricles and atria from SH rats than in those from normotensive rats, whereas the contents of the enzyme activated by cyclic AMP were the same in both groups (Table 1). Since the concentration (0.3 μ M) of either cyclic nucleotide used was such that they activate preferentially the respective protein kinases to a near maximal extent without significantly (<5%) cross activating the others^{8,9}, we interpret the data as evidence that the myocardial levels of soluble G-PK were specifically depressed in SH rats. For comparison, we measured the contents of enzymes activated by cyclic AMP and cyclic GMP in lung extracts from both groups: they were the same. Treatment of SH rats with guanethidine, a drug that depletes catecholamines from peripheral storage, reduced systolic blood pressure and the ratio of heart (ventricle) to body weight due to decreases in cardiac size (Table 2). The drug treatment unexpectedly caused a further depression of the enzyme activated by cyclic GMP, whereas that activated by cyclic AMP remained practically unchanged. The contents of the enzymes in the particulate fraction (30,000g pellet), activated either by cyclic GMP or cyclic AMP, were very low and not affected by hypertension or the drug.

To ascertain that the observations made with crude extracts (Tables 1 and 2) truly reflect alterations in the enzyme levels, G-PK and A-PK in extracts were separated by isoelectric precipitation^{8,9}. Table 3 shows that the myocardial levels of G-PK in SH rats were indeed lower than those in normotensive rats; guanethidine further depressed G-PK, accompanied by decreases in blood pressure. A-PK, on the other hand, was not depressed in SH rats, nor was it affected by the drug. It is interesting that the drug did not affect significantly the G-PK or A-PK levels in the extracts of heart from normotensive rats. The contents of both classes of protein kinases in the extracts of lung of SH and normotensive rats were not affected by the drug (data not shown).

Table 1 Contents of protein kinases stimulated by cyclic GMP and cyclic AMP in extracts of the heart ventricle and atrium and the lung from normotensive and SH rats

Rat and tissue	Content of basal enzyme activity [†]	% Stimulation by		Ratio of stimulation by cyclic GMP to cyclic AMP
		Cyclic GMP	Cyclic AMP	
Heart (ventricle)				
Normotensive (7)	43 $\pm$ 8	91 $\pm$ 4	236 $\pm$ 8	0.38 $\pm$ 0.06
SH (7)	45 $\pm$ 12	24 $\pm$ 2*	230 $\pm$ 7	0.10 $\pm$ 0.02*
Heart (atrium)				
Normotensive (7)	52 $\pm$ 6	80 $\pm$ 7	129 $\pm$ 22	0.62 $\pm$ 0.05
SH (7)	56 $\pm$ 8	33 $\pm$ 8*	115 $\pm$ 16	0.29 $\pm$ 0.02*
Lung				
Normotensive (7)	125 $\pm$ 12	105 $\pm$ 10	196 $\pm$ 12	0.54 $\pm$ 0.04
SH (6)	132 $\pm$ 15	109 $\pm$ 13	195 $\pm$ 16	0.56 $\pm$ 0.06

Systolic blood pressures, measured by the tail cuff method (Arteriosonde 1010, Roche), for the normotensive rat (male, 8 months old) and SH rat (male, 8 months old) were 126 $\pm$ 6 and 220 $\pm$ 12 mmHg, respectively. The ratios of ventricle to body weight for the normotensive and SH rats were 0.0028 $\pm$ 0.0002 and 0.0041 $\pm$ 0.0001, respectively. The rat tissues were homogenised in 10 volumes of ice-cold 50 mM potassium phosphate buffer (pH 7.0), using glass-Teflon homogenisers. Crude extracts were obtained by centrifuging the homogenates for 20 min at 30,000g. The standard assay system^{4,5} for protein kinases contained, in a final volume of 0.2 ml, potassium phosphate buffer (pH 7.0), 10 μ M; theophylline, 0.5 μ M; arginine-rich histone (HA, Worthington), 40 μ g; MgCl₂, 2 μ M; γ -³²P ATP, 1 nmol, containing about 1.1 $\times$ 10⁶ c.p.m.; either cyclic AMP or cyclic GMP, 60 pmol; and appropriate amounts (10–28 μ g) of protein kinase fractions. Crude protein kinase modulator (50 μ g protein) from rat liver was added to the incubation mixture when the cyclic GMP-stimulated enzyme activity (G-PK) was measured, whereas it was omitted when the cyclic AMP-stimulated activity (A-PK) was measured. The reaction was carried out for 10 min at 30 °C. One unit of enzyme is that amount of enzyme activity that transferred 1 pmol of ³²P from γ -³²P ATP to histone in the assay conditions.

[†]Enzyme activity is expressed as U per mg protein in extracts. Numbers of rats used are given in parentheses.

*Significantly different from the normotensive rats ($P < 0.005$).

Table 2 Effects of guanethidine on contents of protein kinases in ventricular extracts from SH rats

Guanethidine treatment	Blood pressure (mmHg)	Ratio of ventricle to body weight	Content of basal enzyme activity†	% Stimulation by Cyclic GMP	% Stimulation by Cyclic AMP	Ratio of stimulation by cyclic GMP to cyclic AMP
Experiment 1						
Untreated (4)	213±8	0.0041±0.0001	48±2	27±3	197±9	0.14±0.01
Treated (4)	162±9*	0.0036±0.0001*	49±2	12±2†	195±13	0.06±0.01†
Experiment 2						
Untreated (5)	189±4	0.0040±0.0001	67±4	26±2	215±26	0.12±0.02
Treated (5)	152±3*	0.0036±0.0001†	75±8	11±3†	207±29	0.05±0.01†

Male SH rats (8 months old) and female SH rats (7 months old) were used for experiments 1 and 2, respectively. Treated rats were given guanethidine (25 mg d⁻¹) orally for 14–19 d, and the rats were killed 24 h after the last drug administration. Assay conditions were as in Table 1.

†Enzyme activity is expressed as U per mg protein in extracts. Numbers of rats used are given in parentheses.

*Significantly different from the non-treated group ($P < 0.001$).

†Significantly different from the non-treated group ($P < 0.005$).

Table 3 Levels of soluble G-PK and A-PK in the heart ventricle of normotensive and SH rats with or without guanethidine treatment

Rat and guanethidine treatment	Blood pressure (mmHg)	Protein kinase activity (U × 10 ⁻³ per g tissue)					
		None	pH 5.3-precipitate Cyclic GMP	Cyclic AMP	None	pH 5.3-supernatant Cyclic GMP	Cyclic AMP
Normotensive rats							
Untreated (5)	122±6	0.7±0.1	2.8±0.3	0.8±0.2	1.6±0.2	2.2±0.2	8.1±0.6
Treated (5)	108±6	0.6±0.1	2.5±0.2	0.8±0.2	1.4±0.1	2.1±0.3	9.5±1.2
SH rats							
Untreated (5)	218±12*	0.4±0.1	1.2±0.2*	0.5±0.1	1.5±0.3	2.5±0.5	8.6±0.7
Treated (5)	152±10†	0.2±0.1	0.6±0.1†	0.3±0.1	1.7±0.2	2.6±0.4	8.5±0.6

Male rats (8 months old) were used in each group. Treated rats were given guanethidine (50 mg d⁻¹) orally for 16 d, and killed 24 h after the last drug administration. G-PK (pH 5.3-precipitate) was assayed in the presence of crude protein kinase modulator (50 µg) whereas A-PK (pH 5.3-supernatant) was assayed in its absence.

Numbers of rats used are given in parentheses.

*Significantly different from the untreated normotensive rats ($P < 0.005$).

†Significantly different from the untreated hypertensive rats ($P < 0.01$).

With perfused hearts⁹ and heart slices¹⁰, cyclic AMP and cyclic GMP may mediate the cardiac stimulatory and inhibitory effects of catecholamines and acetylcholine, respectively. In a state of sustained elevated blood pressure, the heart adapts to work more efficiently. Physiologically, this manifests itself as compensatory hypertrophy. Biochemically, a decreased ratio of the effects mediated by cyclic GMP to those mediated by cyclic AMP may be important. This can be accomplished, at least in part, by either depressing the G-PK levels, or increasing the A-PK levels, or both. Our findings indicate that only the depressed G-PK levels were associated with the cardiac hypertrophy; this may be of some biological significance, since A-PK is present at much higher levels than G-PK and consequently it would require a great deal of the newly synthesised A-PK to lower the enzyme ratio significantly. The effect of guanethidine in further depressing the G-PK levels points to an interesting mode of action of the drug—it may facilitate the compensatory mechanism of the heart. This particular effect of the drug seems to persist even after the hypertension and cardiac hypertrophy of SH rats were largely corrected.

We are investigating whether depression of cardiac G-PK can also be seen in non-genetic forms of chronic hypertension, such as those induced by stress¹¹ and combined administration of deoxycorticosterone and salt¹². It will also be interesting to study the effects of other types of hypotensive drugs, such as minoxidil (a directly acting vasodilator) and clonidine (a centrally acting drug), to determine whether they also cause a further depression of cardiac G-PK in SH rats.

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Possible role for cysteine biosynthesis in conversion from mycelial to yeast form of *Histoplasma capsulatum*

Histoplasma capsulatum is a pathogenic dimorphic fungus which causes histoplasmosis in animals and man. The free-living saprobic form found in soil is mycelial, but, on infection, or in the laboratory, when the temperature is

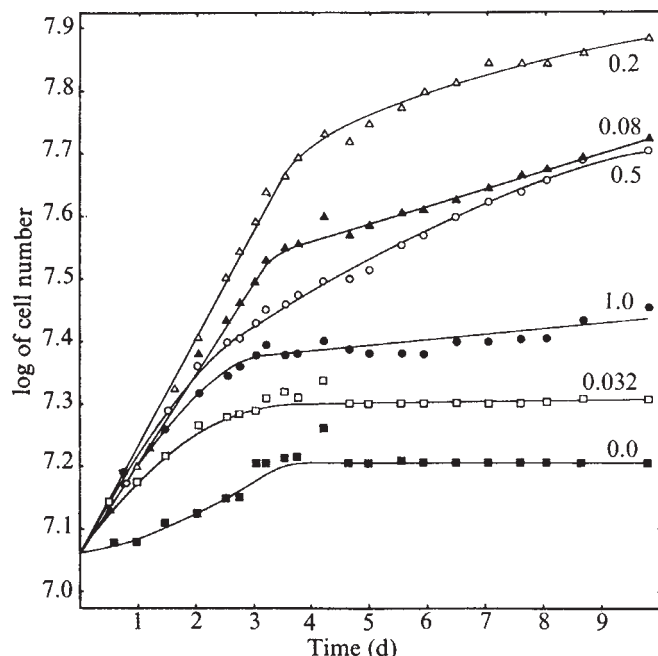


Fig. 1 Effect of increasing L-cysteine concentration on the growth of the yeast phase cells. *Histoplasma capsulatum*, Downs strain, mating type (—), was used throughout this study. Yeast cells (approximately 10^7 cells per ml) were suspended in 50 ml of basal minimal medium (2% glucose, 0.7% Difco yeast nitrogen base without amino acids) in 300-ml triple-baffled flasks with side arms (Bellco). Cysteine was added to final concentrations (mM) as indicated in the graph. The flasks were incubated at 37 °C in a New Brunswick G25 gyratory incubator shaker. The cell growth (turbidity) was measured at 600 nm in a Spectronic 20 colorimeter (Bausch and Lomb).

raised from 23 to 37 °C, it converts reversibly to a unicellular, parasitic yeast-like form. Little is known about the biochemistry of this process. There have been reports of quantitative and qualitative differences in the chemical composition of the two forms^{1,2}, and of changes in RNA metabolism during the conversion³. Also, whereas extracts from the yeast phase cells contain multiple species of RNA polymerase, those from mycelia have only traces of activity, but contain an inhibitor of RNA polymerase⁴. The yeast and mycelial cells differ in sensitivity to amphotericin B (a polyene) and actinomycin D⁵, but there is no significant enzymatic difference between the two phases with respect to the glycolytic pathway and the tricarboxylic acid cycle⁶. The initiation of conversion requires a temperature change and also low oxidation-reduction potential (O/R) of the growth medium⁷, but the maintenance of the yeast phase requires sulphur-containing organic compounds, especially cysteine^{8,9}. The possibility of a nutritional requirement for -SH groups has been suggested¹⁰ but not explored. We now report that the yeast cells lack the enzyme sulphite reductase so that they cannot reduce inorganic sulphite to sulphide, and thus cannot synthesise cysteine. In contrast, mycelial cells, with active reductase, can grow without cysteine supplement.

Figure 1 shows the growth curves obtained for the yeast cells in liquid basal minimal medium to which increasing amounts of L-cysteine were added. At the optimal cysteine concentration (0.2 mM) the generation time in basal medium was about 41 h, about twice as long as in a complex GYE medium (2% glucose, 1% Difco yeast extract). At concentrations higher than 0.2 mM cysteine had a strong inhibitory effect on growth. This could be due to the presence of an excess of reducing groups, or to inhibition of a stage in serine biosynthesis, either by repression or feedback inhibition. This supposition is based on the observed stimulatory effect of serine on yeast growth in the presence

of cysteine, although serine alone does not support the growth (data not shown).

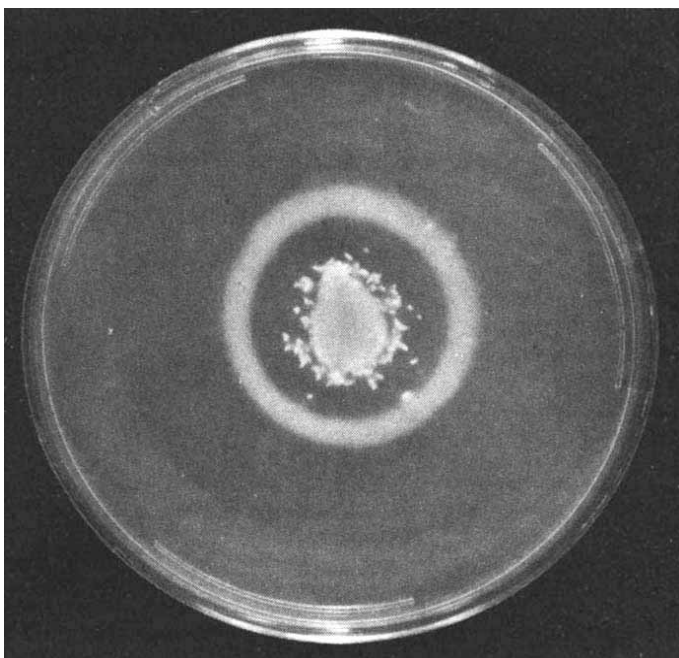
The requirement for cysteine could be satisfied by supplying the cells with Na₂S (Fig. 2). Sodium sulphide is toxic at a high concentration because of the formation of sodium hydroxide when it dissolves. This accounts for the lack of growth and precipitation of cell debris (and also of MgO from the medium) at the point of application of Na₂S crystals to the plate. At a more permissive concentration, however, the sulphide will allow a thick zone of growth to appear.

Cell-free extracts of mycelial and yeast phases were prepared as described before¹¹, and assayed at 37 °C for the ability to reduce SO₃²⁻ (sulphite) to H₂S (hydrogen sulphide). As Fig. 3 shows, the reaction in mycelial extract proceeded in linear fashion with time. In contrast, the yeast extract showed no detectable activity even after 90 min of incubation. Similarly, an increase in protein concentration in the assay resulted in a linear increase in the amount of H₂S produced by mycelial extract, but no activity could be seen in the yeast extract. Lowering the temperature of the assay to 23 °C did not result in the appearance of sulphite reductase activity in the yeast material.

The sulphite reductase activity observed in mycelium was low, about 50–70 times lower than that found in bacterial cells such as *Desulfovibrio vulgaris* (not shown). Nevertheless, the mycelial form of *H. capsulatum* can grow, albeit slowly, on minimal medium without added cysteine. Cysteine stimulates mycelial growth, but its effect is not great. Therefore the slowness of the growth must be due to some other unidentified factor.

It is not entirely surprising that the yeast phase form of *H. capsulatum* is less self-sufficient than the mycelial form. In an infected animal or man all nutrients are available for the fungus to thrive on. It is surprising, however, that the metabolic block is apparently limited to one pathway, and

Fig. 2 Effect of sodium sulphide on the growth of the yeast phase cells. Yeast cells (2×10^7 cells per plate) were plated in a layer of 0.7% agar on a basal minimal medium containing 2% agar. The total solid medium volume in the plate was about 30 ml. A crystal of Na₂S was deposited in the centre of the plate on top of the soft agar layer, and allowed to dissolve and to diffuse into the agar. Almost immediately after the application, small crystals of magnesium oxide started to appear. The plate was taped with Blenderm surgical tape (3M Company), inverted, and incubated at 37 °C for 14 d.



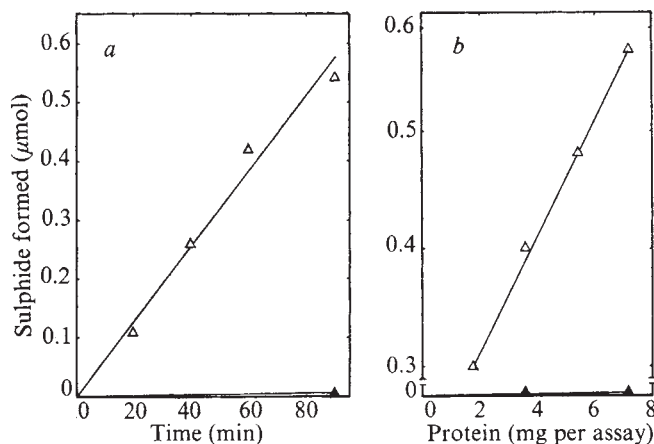


Fig. 3 Sulphite reductase activity in extracts from the mycelial and yeast phase of *H. capsulatum*. Yeast and mycelial cells were grown and extracts prepared as described before¹¹. The extracts (▲, yeast; △, mycelium), containing 10–12 mg protein per ml, were used without further purification for the sulphite reductase assay¹² modified by using Tris buffer, pH 8.0, instead of phosphate buffer. *a*, Activity as a function of time (4.0 mg protein per assay); *b*, activity as a function of protein concentration (time of assay: 60 min.).

one enzyme in that pathway. The lack of sulphite reductase activity in the yeast cells cannot be explained by the thermal instability of the enzyme in the conditions of the yeast phase growth (37 °C). The enzyme from mycelium functions even better at 37 than at 23 °C. Also, the presence in the yeast extract of an inhibitor of sulphite reductase can be excluded because mixing of the yeast and mycelial extracts had no effect on the activity (not shown). It is tempting to hypothesise that the environmental change (temperature rise, drop in the oxidation–reduction potential) triggers biochemical change resulting in a selective shutting-off of a gene for sulphite reductase biosynthesis. The exact mechanism by which the gene inactivation could occur is unknown. One experimental approach to elucidate this mechanism would be to isolate mycelial and yeast mutants unable to undergo morphological transition. The search for such mutants has already been initiated in our laboratory.

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Increased mitochondrial leucyl- and phenylalanyl-tRNA synthetase activity as a result of inhibition of mitochondrial protein synthesis

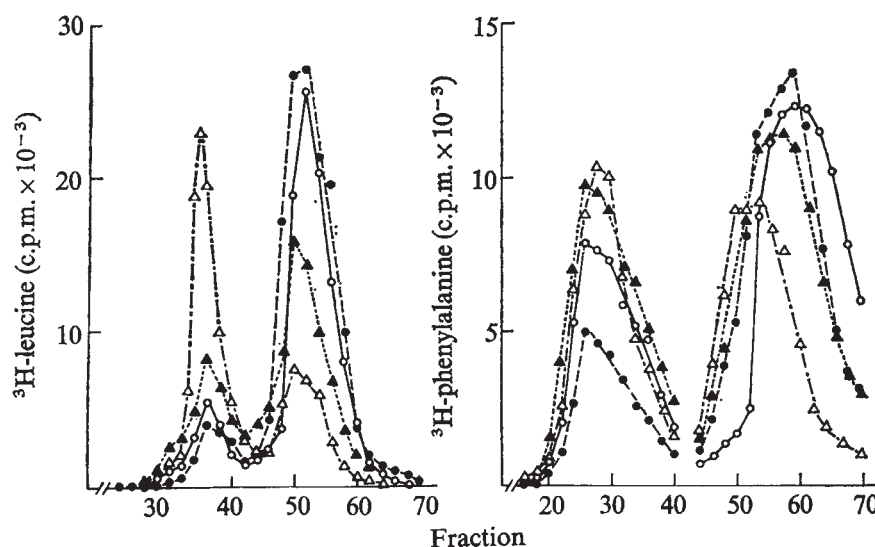
THE synthesis of certain mitochondrial proteins is inhibited by cycloheximide, an inhibitor of cytoplasmic protein synthesis, and not by the inhibitors of mitochondrial protein synthesis, chloramphenicol and ethidium bromide^{1,2}. This suggests that these proteins are synthesised on cytoplasmic ribosomes from messenger RNA (mRNA) transcribed in the nucleus. Their formation, however, is dependent, in some way, on mitochondrial protein synthesis, as growth in the presence of inhibiting concentrations of either ethidium bromide or chloramphenicol results in great increases in the specific activity of mitochondrial DNA-dependent RNA polymerase¹, elongation factors, and methionyl-tRNA transformylase, as well as an increase in mitochondrial ribosomal proteins². This led Barath and Küntzel¹ to propose that the increased synthesis of mitochondrial proteins results from a disruption of mitochondrial control over transcription of nuclear genes specifying the structure of mitochondrial proteins. The model proposes a repressor protein, specified and produced by the mitochondrion, that regulates transcription of mitochondrion-specific genes in the nucleus. When synthesis of the putative repressor is inhibited by either ethidium bromide or chloramphenicol, the target nuclear genes are transcribed at an increased rate and the production of mitochondrial proteins increases. Although a direct test of Barath and Küntzel's model would involve identifying the repressor and its targets, some of the underlying assumptions may be checked by measuring the production by *Neurospora* of the mitochondrial leucyl-tRNA synthetase, whose structure is known to be determined by a nuclear gene in the *Leu-5* region of linkage group V (refs 3 and 4, and our unpublished results). In this case the site of mRNA synthesis is deduced from formal genetic analysis rather than from patterns of inhibition by inhibitors of protein synthesis. As an internal control we also followed the synthesis of the mitochondrial phenylalanyl-tRNA synthetase (whose genetics is unknown). We found that the synthesis of the leucyl-tRNA synthetase in response to chloramphenicol, ethidium bromide and cycloheximide fully conformed to Barath and Küntzel's model. The production of phenylalanyl-tRNA synthetase activity in response to the inhibitors, however, suggests the involvement of either activation of extant enzyme or processing of an inactive precursor molecule as a large component of the increase in the specific activity of the enzyme after treatment with chloramphenicol.

The leucyl and phenylalanyl-tRNA synthetases are easily resolved and quantified by chromatography on standardised hydroxyapatite columns⁵. Figure 1 illustrates the resolution of the mitochondrial and cytoplasmic leucyl and phenylalanyl-tRNA synthetases on hydroxyapatite and the general observation that the amount of mitochondrial enzyme (first to elute from the column in both instances) increases as a function of chloramphenicol inhibition, whereas the amount of cytoplasmic enzyme decreases. Prolonged growth of *Neurospora* in the presence of 7 mg ml⁻¹ chloramphenicol resulted repeatedly in a five- to sevenfold increase in specific activity of the mitochondrial leucyl-tRNA synthetase concomitant with a 50–70% reduction in the cytoplasmic enzyme. In the same circumstances the specific activity of the mitochondrial phenylalanyl-tRNA synthetase was found to increase four- to fivefold, whereas the activity of the cytoplasmic enzyme was reduced by approximately 30%. Similar increases in specific activity of the mitochondrial tRNA synthetases have been obtained

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Fig. 1 Chromatographic resolution on hydroxyapatite of leucyl- (a) and phenylalanyl-tRNA synthetases (b) as a function of growth of *Neurospora* on chloramphenicol. The standard strain STD8A was grown for 20 h in the presence of 0 (●) and 2 mg ml⁻¹ chloramphenicol (○) and for 35 h in the presence of 4 (▲) and 7 mg ml⁻¹ chloramphenicol (△) at 34 °C with aeration in 1.0 l Vogel's synthetic medium. The inoculum was 40 ml of a 1.0 A₅₅₀ conidial suspension. Extraction of mycelia, ammonium sulphate fractionation, hydroxyapatite chromatography and enzyme assays were carried out as described by Weeks and Gross⁵; 52 mg protein were applied to each of the columns of hydroxyapatite. The assay for leucyl-tRNA synthetase activity utilised *Escherichia coli* tRNA, which is charged by both the mitochondrial and cytoplasmic enzymes. The phenylalanine enzymes are quite specific; the mitochondrial enzyme charges only *Neurospora* mitochondrial or *E. coli* tRNA, whereas the cytoplasmic enzyme is very much more efficient in charging *Neurospora* cytoplasmic tRNA than *E. coli* tRNA^{3,5}. For clarity, the data plotted for the phenylalanyl-tRNA synthetases are the charging of *E. coli* tRNA by the mitochondrial enzyme (fractions 16–40) and of *Neurospora* tRNA by the cytoplasmic enzyme (fractions 44–70).



after growth of *Neurospora* in the presence of 10 µg ml⁻¹ ethidium bromide. Since the mode of inhibitory action of ethidium bromide on mitochondrial protein synthesis is quite different from that of chloramphenicol—inhibiting preferentially mitochondrial DNA transcription^{6,7}—the increase in the activities of the mitochondrial tRNA synthetases seems to be a consequence of inhibition of mitochondrial protein synthesis rather than of some specific effect of chloramphenicol.

To ensure that the increase in the mitochondrial leucyl-tRNA synthetase activity really represents an increase in the mitochondrial enzyme rather than some chloramphenicol-induced change in mobility of the cytoplasmic enzyme, the tRNA specificity, antibody sensitivity and electrophoretic mobility of the mitochondrial leucyl-tRNA synthetase from chloramphenicol-treated mycelia were examined and found to be identical to the enzyme obtained from untreated mycelia. In addition, 45208t (*leu-5*⁻), a mutant strain of *Neurospora* shown previously to produce little, if any, active mitochondrial leucyl-tRNA synthetase^{3,5}, failed to yield discernible quantities of the enzyme after growth in the presence of inhibiting concentrations of chloramphenicol. Furthermore, *leu-5*^M, a strain of *Neurospora* which produces an electrophoretic variant of the mitochondrial leucyl-tRNA synthetase (our unpublished results), responds to chloramphenicol inhibition by

producing, in excess, only the *leu-5*^M mitochondrial enzyme.

In spite of severe inhibition of mitochondrial protein synthesis by chloramphenicol, both mitochondrial synthetases were found associated with the mitochondrial fraction. Indeed the specific activity of the two enzymes in the mitochondrial fraction was found to be elevated by a factor nearly equivalent to that of the whole cell extract. Thus, in spite of severe inhibition of mitochondrial protein synthesis by chloramphenicol⁸ at concentrations that are supposed to damage intact mitochondria⁹, proteins find their way to, and become an integral part of, the organelle.

The increase in specific activity of mitochondrial leucyl and phenylalanyl-tRNA synthetases occurs quickly after transfer to medium containing chloramphenicol. The data in Table 1 indicate a two- to threefold increase in the specific activity of the leucyl-tRNA synthetase and a fivefold increase in the phenylalanine enzyme within 4 h. Neither enzyme is elevated during growth in the presence of cycloheximide alone, but the two enzymes respond very differently to combined treatment with chloramphenicol and cycloheximide. The specific activities of the mitochondrial and cytoplasmic leucyl-tRNA synthetases remain at 70–80% of the untreated control in the presence of chloramphenicol and cycloheximide at all concentrations used. Surprisingly, the increase in the specific activity of the mitochondrial phenylalanyl-tRNA synthetase in the

Table 1 Effect of cycloheximide on derepression of mitochondrial tRNA synthetases by chloramphenicol*

Chloramphenicol (mg ml ⁻¹)	Cycloheximide (µg ml ⁻¹)	Mycelia wet weight (g l ⁻¹)	tRNA synthetase specific activities† (% control)		
			Mitochondrial Leucyl	Phenylalanyl	Cytoplasmic leucyl
0	0	9.2	372 (100)	2,790 (100)	4.2 × 10 ⁴ (100)
3	0	5.5	591 (159)	3,555 (127)	4.7 × 10 ⁴ (112)
4	0	4.4	905 (243)	9,056 (325)	3.9 × 10 ⁴ (93)
5	0	3.0	986 (265)	13,913 (499)	4.5 × 10 ⁴ (107)
0	4	3.75	287 (77)	2,869 (103)	3.2 × 10 ⁴ (76)
3	4	2.5	287 (77)	3,478 (125)	2.9 × 10 ⁴ (69)
4	4	1.75	289 (78)	5,217 (186)	3.4 × 10 ⁴ (81)
5	4	1.0	301 (81)	10,909 (391)	3.0 × 10 ⁴ (71)

*Mycelia obtained after growth for 13 h in the absence of inhibitors, as described in Fig. 1, were divided in 3-g portions and resuspended in medium with varying amounts of chloramphenicol with or without cycloheximide. Mycelia were collected after 4.0 h growth without cycloheximide or 4.5 h growth with cycloheximide at 34 °C with aeration.

†The enzymes were resolved by hydroxyapatite chromatography and the active fractions pooled. The specific activities, determined on the basis of initial velocities, are in terms of c.p.m. ³H-aminoacyl-tRNA formed per mg protein per min.

presence of chloramphenicol is not appreciably reduced by cycloheximide. The data suggest that the chloramphenicol-stimulated increase in activity of the mitochondrial leucyl-tRNA synthetase is dependent on cytoplasmic protein synthesis, whereas the increase in the activity of the phenylalanine enzyme is not.

The data of Table 1 also indicate that when both mitochondrial and cytoplasmic protein synthesis are inhibited, extensive degradation of the input mycelia occurs. This is demonstrated not only by a decrease in mycelial wet weight, but also qualitatively by the dark colour and rubbery consistency of the mycelia. Although we find this a troublesome consequence of simultaneously turning off mitochondrial and cytoplasmic protein synthesis, it does not significantly affect our conclusions, as the degradation seems random with respect to the enzymes studied. There is no correlation between weight loss and the specific activity of the leucine enzymes and there is reasonable correspondence between the increases observed in the specific activity of the mitochondrial phenylalanyl-tRNA synthetase in the presence of chloramphenicol with or without cycloheximide.

The pattern of synthesis of the mitochondrial leucyl-tRNA synthetase conforms to Barath and Kuntzel's suggestion of the involvement of a controlling element synthesised in mitochondria that exercises control over the rate of transcription of nuclear genes specifying the structure of mitochondrial proteins. Credence to the model is added, at least as far as the mitochondrial leucyl-tRNA synthetase is concerned, by the fact that the structural gene has been located in the nucleus by conventional genetic analysis (our unpublished results), rather than by inference from patterns of inhibition of synthesis by specific antibiotics. In contrast to the above, the increase of mitochondrial phenylalanyl-tRNA synthetase activity after simultaneous inhibition of mitochondrial and cytoplasmic protein synthesis is incompatible with the model. Barring the existence of a protein-synthesising system insensitive to both cycloheximide and chloramphenicol, it must be assumed that the increase in activity is largely due to removal or decay of some specific enzyme inhibitor or to processing of a precursor of the mitochondrial phenylalanine-activating enzyme. (The precursor may be a component of the cytoplasmic enzyme. The cytoplasmic phenylalanyl-tRNA synthetase does not chromatograph as a homogeneous species¹⁰ and, in addition to reduced specific activity, seems consistently to elute earlier when isolated from mycelia grown in the presence of chloramphenicol.) The extraordinary feature of the system is, however, that whatever the basis for the increase in specific activity of the phenylalanine-activating enzyme it is associated either directly or indirectly with disruption of mitochondrial protein synthesis. It seems likely that in addition to the genetic regulatory molecule(s) proposed by Barath and Kuntzel, which is likely to be involved in regulating the synthesis of the mitochondrial leucine activating enzyme, some component of the mitochondrion is involved in the conversion of the precursor of the phenylalanine enzyme into the active enzyme with appropriate properties of localisation and substrate specificity.

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Evidence for post-transcriptional selection of viral mRNA in cells transformed by human adenovirus 12

CONTROL of gene expression occurs mainly at the transcriptional level in prokaryotic cells¹. In eukaryotic cells, the sites of transcription and translation are physically separated by the nuclear membrane, giving rise to the possibility of regulation by post-transcriptional mechanisms. Control could be exercised at several stages of mRNA biogenesis: methylation, polyadenylation, cleavage, or transport across the nuclear membrane^{2,3}.

The transcription of DNA tumour virus genes provides a useful model for analysing post-transcriptional regulation in eukaryotic cells. The expression of integrated virus genes in transformed cells is thought to be regulated by host cell mechanisms. There is evidence to suggest that in adenovirus-transformed cells viral mRNA formation shares important features with cell mRNA formation⁴⁻⁷. Viral DNA probes of very high specific radioactivity can be prepared to detect both stable cytoplasmic mRNA as well as short lived nuclear viral transcripts. Using such probes, we show here that only a fraction of nuclear viral RNA sequences are exported to the cytoplasm in hamster cells transformed by highly oncogenic human adenovirus 12 (Ad 12).

The Ad 12-transformed hamster cell line, HE C19, contains multiple copies of all or nearly all of the viral genome in an integrated state and different portions of the viral genome are present in equimolar quantities⁸. These cells, therefore, provide a model to study the expression of a specific integrated multigenic sequence. In this study, we determined the fraction of the Ad 12 genome expressed as viral RNA sequences in the nucleus and the cytoplasm of HE C19 cells using *in vitro* labelled Ad 12 DNA probes of very high specific activity. Nuclear, cytoplasmic, and polyribosomal RNA were isolated from HE C19 cells, previously shown to transcribe relatively large quantities of virus-specific RNA⁹. Cellular RNA in vast excess ($2-10 \text{ mg ml}^{-1}$) was annealed with Ad 12 ³²P-DNA ($2 \times 10^8-4 \times 10^8 \text{ c.p.m. } \mu\text{g}^{-1}$) labelled *in vitro* by the "nick translation" reaction of *E. coli* DNA polymerase 1 (refs 8 and 10). The high ratios of RNA to DNA (Table 1) enabled the detection of viral RNA species present in low abundance. These "scarce" RNA sequences are not detected by probes of lower specific activity ($10^6-10^7 \text{ c.p.m. } \mu\text{g}^{-1}$) such as those prepared by *in vivo* labelling (not shown). As shown in Fig. 1, cytoplasmic RNA saturated 10-11% of viral DNA after 6 h of annealing; this value was not increased after 24 h. We conclude that 10-11% of the duplex viral DNA genome representing 20-22% of its asymmetric coding capacity gives rise to cytoplasmic RNA. Similar experiments with RNA isolated from two polyribosome preparations gave identical results—10-12% hybridisation saturation (Table 1). At least 93% of the viral DNA sequences are integrated into the cellular genome⁸. Thus, more than 70% of the potential viral genetic information does not give rise to mRNA.

To determine whether viral RNA sequences are present in the nucleus that are not exported to the cytoplasm, nuclear RNA (2 mg ml^{-1}) was annealed with Ad 12 ³²P-

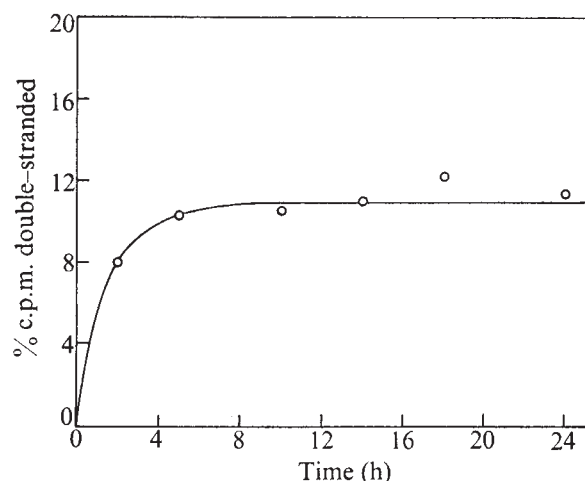


Fig. 1 Hybridisation of *in vitro* Ad 12 ^{32}P -DNA with HE C19 cytoplasmic RNA. Ad 12 viral DNA was prepared as described^{11,12} and labelled with ^{32}P -deoxyribonucleoside triphosphates to specific activities of 2×10^8 – 4×10^8 c.p.m. μg^{-1} by the nick translation reaction of *E. coli* DNA polymerase I (ref. 10) essentially as described by Paul Berg *et al.* (personal communication), scaled down to 4 μl reaction mixture (J.K.M., K. Brackmann, M.R.G., and M.G., unpublished). Heat denatured Ad 12 ^{32}P -DNA (800–1,000 c.p.m.) was annealed at 68 °C with HE C19 cytoplasmic RNA (10 mg ml^{-1}) in 5 μl of 0.72 M NaCl, 10 mM PIPES (pH 6.7), 1 mM EDTA, and 0.05% SDS in sealed siliconised capillary tubes (Fisher). Tubes were removed at the indicated times, cooled to 4 °C, and transferred to a solution containing 30 mM sodium acetate (pH 4.5), 50 mM NaCl, 1 mM ZnSO_4 , 5% glycerol, and 40 μg denatured calf thymus DNA. Samples were split equally into two test tubes and S1 nuclease was added to one of the tubes. The reaction mixtures were incubated for 1 h at 50 °C and the fraction of duplex DNA determined by measurement of trichloroacetic-acid-precipitable radioactivity. The extent of self-annealing of the probe for all time points was determined in reactions substituting 10 mg ml^{-1} of yeast tRNA for HE C19 RNA. The self-annealing background (less than 8% after 72 h) was subtracted from each value. The average of duplicate values are plotted in the figure. To prepare HE C19 RNA, cells were collected, washed twice with phosphate buffered saline, and resuspended in 10 volumes of ice-cold isotonic high pH (Iso-hi-pH) buffer¹³ containing 40 μg ml^{-1} of dextran sulphate. Nonidet P-40 (0.5%) (Shell) was added to lyse cells. All steps were performed at 4 °C. Nuclei were collected by centrifugation at 800g. The cytoplasmic supernatant was precipitated with 2.5 volumes of 95% ethanol and 0.3 M NaCl at –20 °C overnight. The precipitate was collected by centrifugation resuspended in 10 ml of 0.1 M Tris-HCl (pH 9.0) and 0.5% SDS, and extracted three times with equal volumes of water-saturated phenol. The phenol phase was extracted with an equal volume of buffer, the aqueous phases combined, and alcohol precipitated. The pellet was resuspended in 5–10 ml of 0.1 M NaCl, 1 mM MgCl_2 , 0.01 M Tris-HCl (pH 7.4), and incubated with 100 μl ml^{-1} of iodoacetate-treated¹⁴, electrophoretically purified pancreatic DNase (Worthington) for 1 h at 37 °C, followed by three extractions with equal volumes of water-saturated phenol. RNA was precipitated, DNase treated, phenol extracted, and again precipitated. RNA was dissolved in 1 ml of 0.01 $\times$ SSC and passed through a Sephadex G-100 column to remove DNA fragments. The peak of RNA was alcohol precipitated and resuspended in a small volume of 0.01 $\times$ SSC. Polyribosomes were prepared from the cytoplasmic supernatant after removal of

mitochondria by centrifugation at 12,000g for 10 min in a Sorvall at 4 °C. The supernatant was layered on to a discontinuous sucrose gradient containing 2 ml of 2 M sucrose and 2 ml of 1.28 M sucrose made up in Iso-hi-pH buffer plus 100 μg ml^{-1} dextran sulphate. Polyribosomes were collected by centrifugation at 45K in a Spinco Ti50 rotor at 4 °C for 3 h or overnight. The polyribosome pellet was suspended in 10 ml of 0.1 M Tris-HCl (pH 9.0) and 0.5% SDS, and RNA was isolated as described above for total cytoplasm. The absence of viral DNA was confirmed by alkaline hydrolysis (0.2 N KOH, 100 °C, 15 min) of a small portion of the RNA preparations followed by hybridisation to *in vitro* labelled Ad 12 ^{32}P -DNA; in all cases less than 1% hybridisation above self-annealing background was observed after 48 h. S1 nuclease was prepared from *Aspergillus oryzae* α -amylase. The α -amylase was stirred for 1 h at 4 °C in 20 mM sodium acetate (pH 4.5), 0.1 mM ZnSO_4 , 50 mM NaCl, and 5% glycerol, and the precipitate removed by centrifugation. The supernatant was adjusted to pH 5.0, heated to 70 °C with vigorous stirring, and quick-chilled in ice. Following removal of the precipitate by centrifugation, the enzyme was purified by DEAE cellulose chromatography as described¹⁵. The enzyme was stored in 50% glycerol at –20 °C.

DNA. As shown in Fig. 2, 24% of the viral DNA probe hybridised by 24 h, and 38% by 84 h. Since saturation was not reached at this time, 38% is a minimal estimate of the viral sequence content of nuclear RNA. Analysis of a different nuclear RNA preparation showed 31% hybridisation at 24 h (Table 1). Several factors may account for the inability of nuclear RNA to saturate the viral DNA probe: (1) some viral RNA species may be present in very small amounts; (2) RNA is degraded during long hybridisation periods; (3) RNA–RNA hybridisation of putative symmetrical RNA transcripts may compete with the RNA–DNA hybridisation reaction.

Highly radioactive viral DNA probes could detect small amounts of viral DNA that may contaminate RNA preparations. The following precautions eliminate this possibility. Nuclear and cytoplasmic RNAs were digested two or three times with pancreatic DNase which in our hands destroys all viral DNA even in RNA preparations from productively infected cells; the latter contain 10^5 times more viral DNA than do transformed cells. Diphenylamine analysis on

nuclear RNA (800 μg) detected less than one-tenth the amount of viral DNA necessary to drive the hybridisation reaction. Alkaline hydrolysis of nuclear RNA eliminated hybridisation to viral DNA (see legend to Fig. 1).

This is the first demonstration to our knowledge that viral RNA sequences are present in the nucleus of a transformed cell which are not transported to the cytoplasm. It is possible that the non-mRNA sequences in the nucleus arise through errors in transcription, and that the expression of integrated Ad 12 genes is primarily regulated at the transcriptional level. Even so, our data suggest that strong post-transcriptional controls must operate to transport selectively mRNA sequences to the cytoplasm, and to restrict non-mRNA sequences to the nucleus. This is analogous to mRNA biogenesis in uninfected eukaryotic cells in which most nuclear RNA sequences do not reach the cytoplasm^{3,16,17}. Our data provide preliminary evidence for post-transcriptional regulation of gene expression as distinguished from RNA processing since nearly all the Ad 12 genome specifies mRNA, that is late after productive

Table 1 Hybridisation of HE C19 with *in vitro* labelled Ad 12 ^{32}P -DNA

HE C19 RNA	Ad 12 ^{32}P -DNA	RNA/DNA	Longest hybridisation time (h)	Largest $R_{\theta t}$	% DNA hybridisation
1 Cytoplasm (prep 1)	Probe 1 (2×10^8 c.p.m. μg)	1.2×10^7	24	2,880	11
2 Polysomal (prep 1)	Probe 1 (2×10^8 c.p.m. μg)	7.5×10^6	48	3,458	12
3 Polysomal (prep 2)	Probe 2 (3.5×10^8 c.p.m. μg)	1.3×10^7	25	1,800	10
4 Nuclear (prep 1)	Probe 1 (2×10^8 c.p.m. μg)	2.5×10^6	84	2,016	38
5 Nuclear (prep 2)	Probe 3 (2.5×10^8 c.p.m. μg)	3.1×10^6	24	576	31

RNA was prepared as described in the legends to Figs. 1 and 2.

Cytoplasmic RNA (10 mg ml^{-1}), polysomal RNA (6 mg ml^{-1}), or nuclear RNA (2 mg ml^{-1}) were annealed using standard hybridisation conditions described in Fig. 1. Reactions were stopped at the specified times and assayed for ^{32}P -DNA hybridised. The value for the last time points are given in this table.

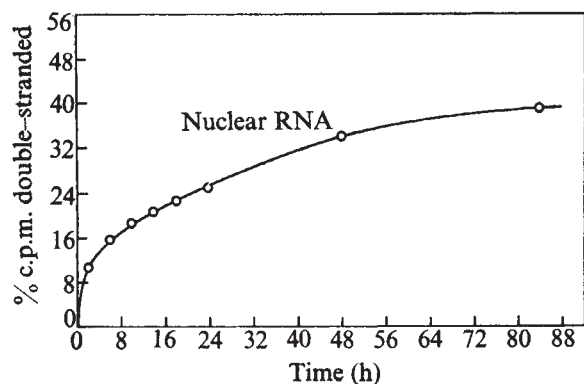


Fig. 2 Hybridisation of *in vitro* Ad 12 ^{32}P -DNA with HE C19 nuclear RNA. Nuclei were collected as described in the legend to Fig. 1 and washed twice with 10 volumes of Iso-hi-pH buffer plus one-tenth volume of detergent mix (2 parts 10% Tween-40, 1 part 10% deoxycholate). The pellet was resuspended in 10 volumes of 0.05 M sodium acetate, 0.01 M EDTA, pH 5.1 and 0.5% SDS, dispersed with a Dounce homogeniser, and extracted with an equal volume of water-saturated phenol plus one half volume of chloroform-isoamyl alcohol (24:1) at 68 °C for 3 min. The phenol phase was extracted with one half volume of Iso-hi-pH buffer at 4 °C. The aqueous phases were combined and extracted with an equal volume of water-saturated phenol and one half volume of chloroform-isoamyl alcohol at 4 °C. RNA was precipitated, dissolved in 10 ml of 0.1 M NaCl, 1 mM MgCl_2 , 0.01 Tris-HCl (pH 7.4), and DNase treated, phenol extracted, and precipitated. The DNase treatment was repeated twice and the RNA further purified as described. Alkaline hydrolysis was performed on all RNA preparations; less than 1% hybridisation above self-annealing background was observed after 48 h. Hybridisation was performed as described in the legend to Fig. 1 except that 2 mg ml^{-1} of nuclear RNA was used instead of 10 mg ml^{-1} of cytoplasmic RNA.

infection, polyribosomal RNA saturates 48–49% of Ad 12 ^{32}P -DNA, representing over 95% of the asymmetrical coding capacity of the viral genome (unpublished data). By contrast, most of the eukaryotic genome does not code for mRNA^{3,17,18}. Our data do not, however, exclude the formal but unlikely possibility that the sequences we see in the nucleus but not in the cytoplasm represent anti-mRNA from about 60% of the viral genome that never exit even during lytic infection.

Our data provide no direct evidence that primary RNA transcripts are larger than viral mRNA molecules and do not distinguish between two possibilities: that the large primary RNA transcripts are cleaved and processed to smaller ones that are exported to the cytoplasm; or that discrete smaller RNA transcripts are generated, but only some of these are transported to the cytoplasm. Recent studies have suggested that during late stages of productive Ad 2 infection, primary transcripts are large polycistronic molecules derived from more than half of the viral genome, and that these exhibit a precursor-product relationship with smaller cytoplasmic mRNA¹⁹. It is not known, however, whether the transcription of the Ad genome late in productive infection resembles transcription of host cell DNA and integrated viral genes. Further studies with the Ad 12-transformed cell system provide the opportunity to use highly radioactive restriction fragments to study the "discarded" nuclear sequences. An analysis of the properties of the nuclear RNA molecules containing these sequences will be most useful in evaluating the significance of post-transcriptional mechanisms in the primary route of eukaryotic mRNA biogenesis.

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Template-directed synthesis of high molecular weight polynucleotide analogues

A GREAT deal is known about the structures of nucleic acids and the mechanisms involved in nucleic acid replication. This relatively new knowledge has led a number of authors to speculate that other linear heteropolymers might be able to serve as carriers of genetic information. A number of polynucleotide analogues have been synthesised by chemical or enzymatic methods^{1–7} and one of them has been used as a template for RNA polymerase⁴. Two studies, in particular, have some relationship to the present work. 3'–5'-Linked polynucleotides derived from 2'-amino-2'-deoxynucleoside 5'-phosphates have been synthesised enzymatically⁷. Letsinger *et al.*^{5,6} reported the chemical and enzymatic formation of polydeoxyribonucleotides containing N⁶–O^{3'}-phosphoramidate linkages. Here we discuss nucleic acid analogues derived from 2'-amino-2'-deoxynucleotides that we believe may be capable of non-enzymatic replication, and describe some experimental results which, although they are far from establishing the principle of self-replication, do show that it is possible to synthesise fairly long oligomers on templates in aqueous solution.

Polynucleotides have been shown to act as templates for the non-enzymatic condensation of complementary mononucleotides and oligonucleotides⁸. Our own work indicates that, whenever ribonucleotide derivatives are used in template-directed reactions, 2'–5' linkages are formed in excess over 3'–5' linkages. Furthermore, we have found it difficult to achieve an efficiency of condensation greatly in excess of 50% (ref. 9). These observations suggest that a much more effective template-directed reaction could be achieved by using, instead of ribonucleotides, derivatives in which the 2'-hydroxy group is replaced by a more nucleophilic group, for example an SH, NH₂ or NHOR group. So far our experimental work has been restricted to the 2'-amino-2'-deoxynucleotides.

We established first that 2'-amino-2'-deoxyuridine (UNH_2)¹⁰ condenses effectively with uridine 5'-phosphorimidazole (ImpU) or adenosine 5'-phosphorimidazole (ImpA) in

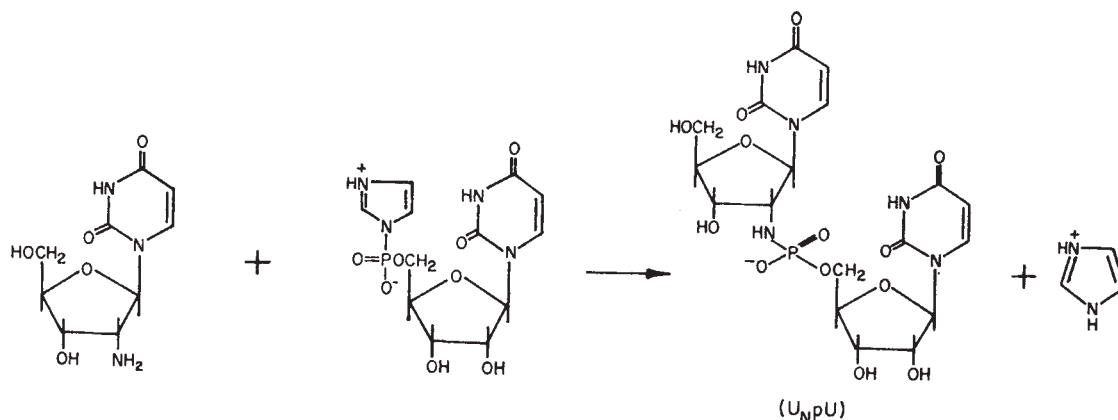


Fig. 1 Condensation of U_{NH₂} with ImpU (or ImpA) to give dinucleoside phosphates.

aqueous solution to give dinucleoside phosphates as shown in Fig. 1. High voltage electrophoresis of the product obtained with ImpA establishes that it has zero charge at pH 2.7. This is consistent with a 2'-5'-phosphoramidate structure and incompatible with a 3'-5'-linked phosphodiester carrying a free 2'-amino group, since a compound with the latter structure would carry a positive net charge at pH 2.7. The presence of a phosphoramidate bond in our product is further indicated by its hydrolysis in 0.1 M formic acid at room temperature^{5,6} ($t_{1/2}$ 70–80 h). The hydrolysis of adenylyl-[5' → 2']-2'-amino-2'-deoxyuridine (U_NpA) at various pHs will be discussed in another paper¹¹.

In aqueous imidazole buffer at pH 7.5 and 0 °C, U_{NH₂} (ref. 10) reacts with ImpA or ImpU 50–100 times faster than does

uridine (U). Yields of U_NpA of about 80% were obtained from solutions of 0.1 M in U_{NH₂} and 0.01 M ImpA. Similar yields of uridylyl-[5' → 2']-2'-amino-2'-deoxyuridine (U_NpU) were obtained in the reaction of U_{NH₂} with ImpU. In identical conditions, uridine reacted with ImpA and ImpU to give at most a few per cent of dinucleoside monophosphate¹¹.

A preliminary experiment with 2'-amino-2'-deoxyuridine 5'-phosphorimidazole (ImpU_{NH₂}) showed that the enhanced reactivity of the amino group also facilitates the synthesis of oligomers. We prepared a concentrated solution of ImpU_{NH₂} (about 0.8 M) in 2.0 M imidazole buffer. After keeping the reaction mixture for 28 days at 0 °C we obtained a 62% yield of a complex mixture of oligomers of 2'-amino-2'-deoxyuridine 5'-phosphate (pU_{NH₂}) including 11.9% moving slower than the

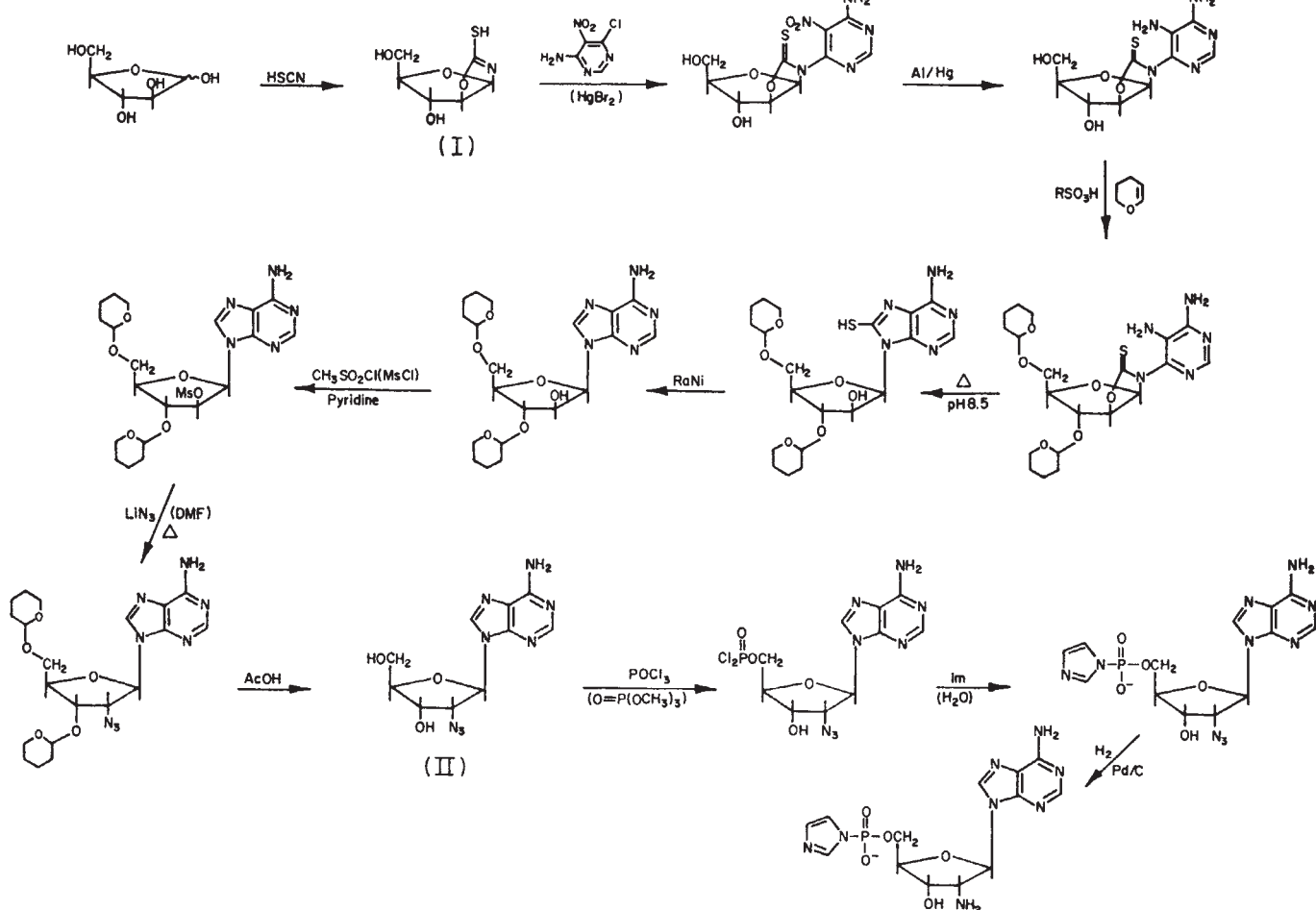


Fig. 2 Scheme for synthesis of ImpA_{NH₂}.

pentauridylylate (Up)₅ on chromatography in *n*-propanol-ammonia-water. In the same conditions ImpU gave very little high molecular weight material.

The previous demonstration that template-directed reactions are much more efficient than reactions in free solution, and yield 2'-5'-linked products, together with the present finding that a 2'-amino group is 50-100 times more reactive than a 2'-hydroxy group in the absence of a template, led us to synthesise 2'-amino-2'-deoxyadenosine 5'-phosphorimidazolide (ImpA_{NH₂}). This enabled us to study the reactivity of a 2'-amino group in a template-directed condensation on polyuridylic acid (poly(U)).

We prepared ImpA_{NH₂} by the route outlined in Fig. 2. The key compound, 2'-azido-2'-deoxyadenosine (II) was obtained in an overall yield of about 8% based on arabinofurano-thionoxazolidine (I). It melted at 215-216 °C (w. dec.). The ultraviolet spectrum of II (λ_{max} at pH 1.0, 257 nm ($\epsilon = 14,900$); at pH 6.4, 259 nm ($\epsilon = 15,000$); at pH 12.0, 258 nm ($\epsilon = 14,900$)) and the nuclear magnetic resonance spectrum were consistent with the proposed structure. Furthermore, II was reduced catalytically to 2'-amino-2'-deoxyadenosine, which had a specific optical rotation of $[\alpha]_D^{25} = 65.1^\circ$ (0.50%, methanol). The reported value for $[\alpha]_D^{25}$ is $-66 \pm 2^\circ$ (ref. 12). We are indebted to Dr R. Ranganathan (ref. 13) for drawing to our attention his novel synthesis of ara A and his preliminary work on the protection of the hydroxyl groups of (I) with tetrahydropyranyl groups.

A solution containing poly(U) (0.05 M), ImpA_{NH₂} (0.025 M), MgCl₂ (0.075 M) and NaCl (0.2 M) was maintained at 0 °C and pH 7.5 for 16 d. The reaction mixture was treated with pancreatic ribonuclease to degrade the poly(U); the resulting solution was loaded on a RPC-5 column and eluted with a KCl gradient. In Fig. 3 we show a record of the ultraviolet absorption (254 m μ) of the eluate.

In independent experiments we found that 2'-5'-linked oligomers (pA_N)_n of 2'-amino-2'-deoxyadenosine 5'-phosphate (pA_{NH₂}) with $n = 1-6$ move slightly behind the corresponding 3'-5'-linked oligomers of adenosine 3'-phosphate on paper chromatography in *n*-propanol-concentrated ammonia-water (55 : 10 : 35). We desalted the materials corresponding to early peaks from the column, and subjected them to chromatography in that system. In this way we were able to estimate the chain length of the early peaks. This enabled us to conclude that the last detectable peak from the column corresponded to an oligomer about 35 units long. The total yield of material in dimer and oligomers amounted to about 87% of the reacted ImpA_{NH₂}.

Control experiments in which the poly(U) template was

omitted, showed that substantial amounts of dimer and some trimer are formed, but only very small amounts of higher oligomers. Thus the presence of a poly(U) template facilitates the synthesis of (pA_N)_n up to the 35-mer in conditions of dilution in which no high molecular weight material is detected in the absence of a template.

The non-catalysed condensation reactions of activated nucleotides are very inefficient in the absence of templates, and only moderately efficient in their presence. Our experiments show that the corresponding reactions of activated 2'-amino-2'-deoxynucleotides are much more efficient. In concentrated aqueous solution complex mixtures of moderately long oligomers are formed, whereas efficient template-directed condensations occur on suitable polymers in fairly dilute solution to give (pA_N)_n. This naturally raises the question of the relevance of the analogues to the origins of life. Could a replicating system have evolved from activated aminonucleotides, and only later have been replaced by the present system of nucleic acids?

It is easy to formulate schemes for the synthesis of 2'-amino-2'-deoxyribose from ribose or ribulose and ammonia (reviewed in ref. 14), but we have been unable to obtain appreciable yields of the amino sugars in this way. Furthermore, our attempts to obtain aminonucleosides from amino sugars in prebiotic conditions were unpromising. Since we did not make extended studies of the reactions we cannot exclude the possibility that a prebiotic synthesis of amino nucleotides is possible. Until such a synthesis is reported, however, we will concentrate on 'non-prebiotic' studies of the reactions of aminonucleotides.

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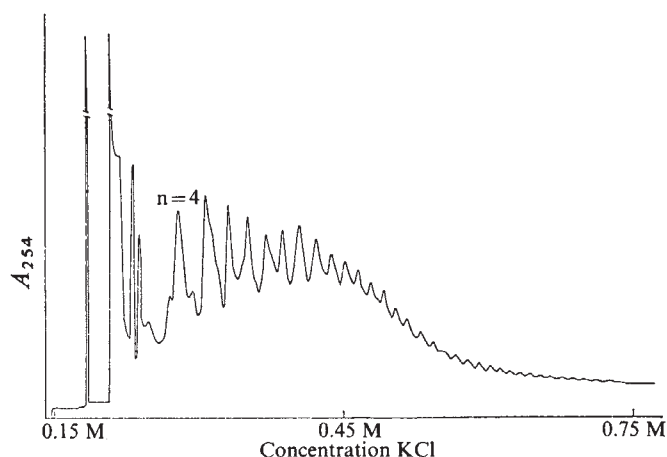
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Fig. 3 Products from poly(U)-directed condensation of ImpA_{NH₂}. The trace is the record of the ultraviolet absorbance at 254 nm of the eluate from a RPC-5 column.



Homology relationships among the small blue proteins

COMPARATIVELY few blue proteins, all containing copper, are found in nature. Isolated from organisms all along the evolutionary scale they are¹ the 'blue oxidases'—laccase and ascorbate oxidase of plant origin and ceruloplasmin (ferroxidase) from mammalian serum (molecular weights 60,000-140,000, 4-8 Cu mol⁻¹)—and the 'small blue proteins'—azurins from bacteria, plastocyanins from photosynthetic cells and others like stellacyanin and umecyanin, found in higher plants (molecular weights, carbohydrate excluded, 11,000-14,000, 1 Cu mol⁻¹). The small blue proteins may all function as electron carriers although a precise role is known only for the plastocyanins.

The so-called "blue" or "type 1" cupric copper in these proteins has been well characterised by spectroscopic methods², and is always found to have the same general properties, not mimicked by any low molecular weight complexes. These are a typical optical spectrum with λ_{max}

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6	--	V	E	V	L	-	G	D	S	-	A	-	V	-	G	N	F	S	V	P	S	-	E	K	I	V	F	K	-	A	G	F	-	V	-	E	D	E	I	-	S	G	V	D	A	A	K	I	-	M	S	E	E	D	L	-	N	A	P	-	E	T	Y	K	V	T	L	--	T	E	-	K	-	T	K	F	Y	S	-	Q	-	V	K	V	-	N	-											
7	-	D	V	T	Y	K	-	A	D	S	-	A	-	V	-	E	S	S	V	T	I	K	A	-	A	-	E	T	V	T	W	-	A	G	F	-	I	-	E	D	E	V	-	S	A	G	N	A	E	A	L	-	--	H	E	D	Y	-	N	A	P	-	E	S	Y	S	A	K	F	--	D	T	-	A	-	T	G	Y	F	E	-	Q	-	K	T	I	-	Q	-									
8	E	T	Y	T	Y	K	-	S	D	-	K	-	L	-	V	-	E	A	K	L	T	I	K	P	-	D	T	V	E	F	L	-	K	V	P	-	V	-	A	A	L	N	-	A	K	S	A	D	L	A	K	-	L	S	H	K	Q	L	M	S	P	-	Q	S	T	S	T	T	F	P	A	D	A	P	E	-	T	F	Y	E	-	R	-	V	-	K	I	-	Q	-								

Fig. 1 Amino acid sequences for the 9 azurins and 8 plastocyanins considered, as collected from the literature. Amino acid residues are represented by their one-letter symbols⁶. Blank indicates that the residue is the same as in the first row and hyphen indicates a deletion. In the azurins Cys 3 and Cys 26 are connected by a disulphide bridge. Sources and references are as follows: Azurins (ref. 3 and R. P. Ambler, personal communication), 1, *Pseudomonas aeruginosa* P6009; 2, *P. denitrificans* NCIB 9496;

3, *P. fluorescens* B-93; 4, *P. fluorescens* C-18; 5, *P. fluorescens* D-35; 6, *Bordetella bronchiseptica* NCTC 8344; 7, *Alcaligenes denitrificans* NCTC 8582; 8, *A. faecalis* NCIB 8156; 9, *Alcaligenes* spp. Plastocyanins, 1, Potato (*Solanum tuberosum*)¹⁸; 2, French bean (*Phaseolus vulgaris*)¹⁷; 3, Broad bean (*Vicia faba*)¹⁸; 4, Elder (*Sambucus niger*)¹⁹; 5, Vegetable marrow (*Cucurbita pepo*)²⁰; 6, Spinach (*Spinacea oleracea*)²¹; 7, Green algae (*Chlorella fusca*)⁴; 8, Blue-green bacteria (*Anabaena variabilis*)²².

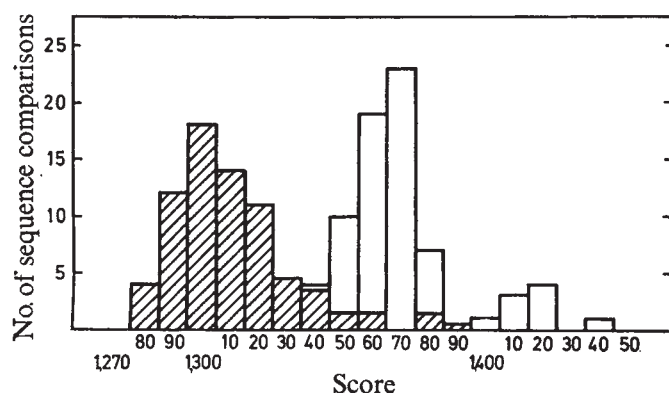


Fig. 2 Distribution of scores for best alignment of azurins with plastocyanins (72 pairs) before (open) and after (cross hatched) random permutations. Two random permutations were done for each pair, but the frequency numbers above are reduced by half to make the two distributions comparable. All bars, also overlapping, start at the bottom of the diagram.

at 610 nm having a molar extinction coefficient around 4,000, a very small hyperfine constant in the electron spin resonance spectrum and a high, albeit varying, redox potential. This indicates that the copper environment is very similar in all of them. This situation—if true—can be the result of either convergent evolution, where similar requirements have promoted the evolution of the same structure from different origins, or divergent evolution, where several or all of these proteins have a common

ancestor. We have attempted to discriminate between these possibilities for the small blue proteins by comparing amino acid sequences that have recently become available for azurins and plastocyanins and, to some extent, for stellacyanin and umecyanin. The results suggest that all the small blue proteins are monophyletic in origin and indicate some of the conserved amino acid residues as potential copper ligands. The apparent similarity between the azurin and plastocyanin primary structure around the single cysteine residue has been noted before^{3,4}.

The nine pseudomonad azurin sequences plus six higher plant, one blue-green alga and one blue-green bacterium (prokaryote) plastocyanin sequences considered are shown in Fig. 1. The azurins have 128–129 amino acids, the plastocyanins 98–104 amino acids. The single disulphide bridge in the azurins is not found in the plastocyanins. Alignment of sequences and score for best alignment was obtained with a computer procedure based on the Needleman and Wunsch³ algorithm. Weights for amino acid pairs, intended for detection of distant relationships, were taken from ref. 6.

Scores were calculated for all 72 possible pairs of azurins with plastocyanins plus two random permutations for each of those pairs. The distributions obtained are shown in Fig. 2; means and standard deviations are reported in Table 1. The distributions for the experimental and random sequences are different at a level of significance of 10^{-80} (assuming Gaussian shape). The nine values in both populations that deviate most from their respective means involve the prokaryotic plastocyanin comparisons, for

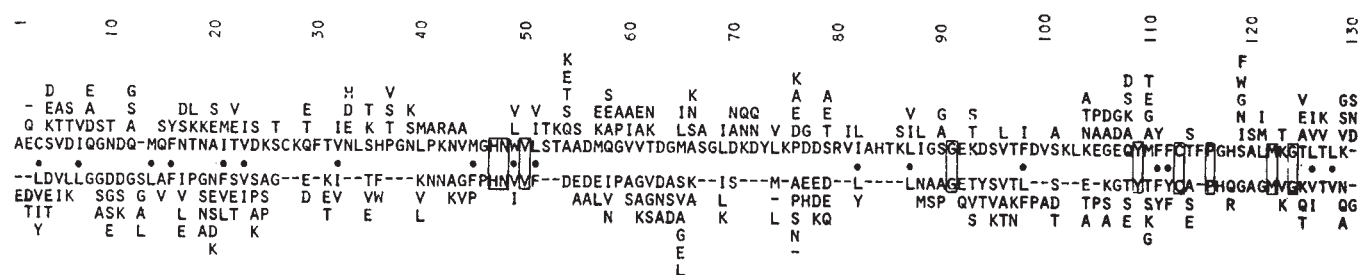


Fig. 3 Alignment of a composite azurin (above) and composite plastocyanin (below) amino acid sequence. Each position in the composite sequence contains all residues observed there in the

individual sequences (Fig. 1). Unvaried residues are shown in boxes whereas conserved aromatic or hydrophobic character is marked with dots.

which no correction was made for its five extra amino acids.

The deletion patterns in the shorter plastocyanins in the above alignments varied considerably although about 80% of the deletions were found in the middle third. A specific deletion pattern was obtained by comparing a composite azurin with a composite plastocyanin sequence. In these each position contained, regardless of their original frequencies of occurrence, one of each amino acid residue observed there. Weights for pairs were taken as the arithmetic mean of the weights for all possible pairs. The composite sequences and their alignment are shown in Fig. 3. Out of the 130 positions, nine amino acids are invariant and an additional 17 have conserved their hydrophobic or aromatic character. Gaps in the plastocyanin sequence are concentrated in two regions. Of the 25 deleted residues 12 are found in position 67–86 and 8 in position 27–39. No gap penalty was used which means that the number of gaps has been maximised. A reasonable gap penalty (2–5) will move residues 29, 31, 32, 69, 70, 74 and 82 and remove up to five gaps, but not influence the alignment around the more conserved regions 47–51 and 109–130.

The score for the alignment of the composite sequences was 7.3 s.d. above the mean of the scores for 20 randomisations. When the 72 pairwise comparisons of the azurins to the plastocyanins, together with two randomisations (compare Fig. 2), were repeated imposing the deletion pattern of Fig. 3, the means of the two distributions were now 200 units apart compared with the earlier 60 (Table 1). About 60 points in the earlier pairwise comparisons were a result of "better than real" alignment. The distribution of similarity along the aligned composite sequences was obtained as normalised score differences, before and after 20 randomisations, for all possible spans of 11 residues. The mean deviation from random was 2.8 s.d. units, with a s.d. of 1.1 s.d. units. Highest deviation from random, about 4 s.d. units, was found around residues 15–25, 42–51 and 105–115.

The similarities found above, although very suggestive, do not by themselves prove the azurins and the plastocyanins to be homologous. A direct test for divergence was carried out using the most parsimonious phylogenetic tree, calculated according to Moore *et al.*⁷, for the proteins in conjunction with the Fig. 3 alignment. The above-chance occurrence of identical nucleotides in the independently constructed sequences found by the parsimony procedure of Fitch⁸ and Fitch and Farris⁹ at the most ancestral node of each of the two groups of proteins, were then calculated¹⁰. The figure obtained, +6.9 s.d., strongly supports the possibility of divergent evolution.

Only fragmentary data are available for the other small blue proteins. Stellacyanin (C. Bergman and L. Strid, unpublished) and umecyanin¹¹ have about 110 and 130 amino acids with a single cysteine residue plus one disulphide bridge. This bridge is not, however, positioned as the one found in the azurins as no cysteine is found in the first 30 positions in umecyanin, nor in the first 12 positions in

Plastocyanins	1	--LDVLLGGDDGSL
	2	--LEVL SGD S
	3	--VEVL ASD G
	4	--VEIL GED S
	5	--IEVL GDD S
	6	--VEVL GGD S
	7	-DVTVK ADS A
	8	ETTVK SDK L
Umecyanin		EDYDV--GGDDE
Stellacyanin		TVYTV--GDSAGWK

Fig. 4 N-terminal amino acid sequences of plastocyanins (Fig. 1) as compared with umecyanin¹¹ and stellacyanin (C. Bergman and L. Strid, unpublished).

stellacyanin. Also, they both have a considerable amount of bound carbohydrate. Their N-terminal sequences nevertheless show considerable similarities with each other and with the N-terminus of the plastocyanins (Fig. 4), indicating that possibly all the small blue proteins have a common origin.

The only known common function for the proteins considered is to bind copper in a very particular way. Residues involved in copper binding are presumably untouched by evolution and to be found among the unvaried (and hydrophilic) sidechains. These are (Fig. 3) His 47, Asn 48 and Cys 113. In addition His 118 in the azurins is unvaried but displaced one position in the plastocyanins. The unvaried Tyr 109 and Met 122 are possible but poor Cu ligands. Spectroscopic data^{12–14} indicate that both nitrogen and sulphur are ligand atoms and chemical evidence¹⁵ points to Cys 113 as one of the ligands.

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Table 1 Means for alignment scores for the azurins compared with the plastocyanins

	Real sequences		Random sequences	
	Mean score	s.d.	Mean score	s.d.
All sequences (72 pairs)*	1,375	20.5	1,316	21.6
All sequences except prokaryotic plastocyanin (63 pairs)*	1,370	9.6	1,322	11.7
All sequences. Given deletion pattern† (72 pairs)	1,318	27.9	1,117	31.0

* Optimal alignment.

† Alignment that is optimal for composite sequences (Fig. 3).

Scores were calculated before and after random permutation of one of the two sequences in a pair. Randomisation was done twice for each pair.

matters arising

Trapa natans in the British Flandrian

I WOULD like to make a palaeobotanical comment and to cite some further literature in reference to the paper by Flenley *et al.*¹

The distribution of isolated post-glacial subfossil finds of *Trapa* (their Fig. 2) can be supplemented by three pollen finds from southern Norway (east of Oslofjord²) and a macrofossil (fruit) find from just north of Gävle in eastern Sweden³ (61°N). At another, central Swedish site⁴ pollen of *Trapa* was found to occur a little higher up the profile than fruits.

All these sites lie within the hypothetical climatic limit for *Trapa* during the climatic optimum (Atlantic and/or Sub-Boreal chronozones) drawn by Hintikka (ref. 5, Fig. 46), based on an extension of the present-day limits (mean temperature of the warmest month >17.5 °C and of the coldest month <1 °C) by Iversen's⁶ postulated increase in mean July temperature of +2 °C. These values are corroborated by the meteorological data for subfossil and recent localities tabulated by Apinis⁷, who in addition draws attention to the critical period in May–June, during which a water temperature on the lake bottom of >12 °C is necessary for seed germination, and of September–December temperatures of 10 °C–1 °C for successful fruit ripening. Hegi (Flenley's ref. 3) states that flowering does not occur at water temperatures <20 °C.

Hintikka's fossil map only covers Fennoscandia, but comparison of the course of the 16 °C mean July isotherm⁸ (interpolated for Fig. 9.2a) for recent time for Britain and western Denmark–southern Norway supports the possibility of subfossil occurrences of *Trapa* in Britain south of the Humber–Dee line (18 °C) in favourable aquatic habitats.

Habitat suitability needs stressing, however, particularly for outpost sites, as regards lake water depth, topography, sedimentation and water chemistry^{9,10}. We are told too little about the site conditions at the Skipsea meres to draw any conclusions; or about any associated members of the aquatic flora (supporting macrofossil studies are required urgently!). The absence of subfossil finds of *Trapa* pollen or fruits at Hockham¹¹ or any other East Anglian meres could con-

ceivably arise from their (generally lime-rich) eutrophy (compare the frequent finds of *Najas marina* seeds and Flenley's ref. 1, pp.100–102), or that they do not lie on swan/goose migration routes, although human influence during the Neolithic and Bronze Age periods would surely have secured the introduction of *Trapa* as easily there as on Humberside? The best correlation between prehistoric sites and former *Trapa* lakes has been presented by Sundelin's¹² investigations in Småland, central southern Sweden, in which the absence of *Trapa* fruits from lakes to the north of his area was ascribed to climatic causes. Comparison with Hintikka's map shows a postulated negative 'inlier' in just that position. *Trapa* and man were obviously coeval there, but without ¹⁴C datings it is hard to be certain that man got there first.

Although not disagreeing with Flenley that man may have had a 'finger in the *Trapa* pie' (although the 'Swan-maiden legend' would explain both the Humberside and South Uist finds!), I cannot agree that "in which case it would not be necessary to invoke climatic change"; a more favourable local climate than that of the present day is a prerequisite for successful establishment and continued presence of *Trapa*. It is an annual and I can find no reference to overwintering by turions, only as fruits, although these remain viable for several years at least.

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FLENLEY AND MALONEY REPLY—We thank Tallantire for his valuable comments¹, and we are sorry that he read our paper to imply that we were trying to explain all the northern European Holocene records as resulting from human activity. As we said originally

"explanations relating to man, however, deserve re-examination in the context of the new records".

In attempting to explain the new British records, we should like to make the following points. First, it is a general principle in ecology that a species cannot be assumed to occupy its full potential range in the field². The entire ecological amplitude can only be established by comprehensive laboratory experiments. Although the experiments of Apinis³ undoubtedly go some way towards this, they seem to relate more to specific phases (for example, germination, fruit ripening) than to the complete life cycle. Cultivated plants commonly occur well outside their 'wild' range.

Second, ecotypic differentiation may be expected to occur rather rapidly in an annual species, and the possibility of an extinct ecotype cannot be eliminated. Even in perennial species, ecotypic differentiation is very rapid⁴.

Third, we have investigated, in addition to the two meres which did contain both *Trapa* pollen and Bronze Age dwelling platforms, four other north Humberside former meres from which Bronze Age dwelling platforms have not been reported, and we did not find *Trapa* pollen in any of these. Of course, it is difficult to argue from correlations based on absence, which is why we did not adduce this evidence in the original letter. We note, however, that Tallantire refers to the absence of finds of *Trapa* in East Anglian meres.

Fourth, there seems to be some similarity between the Bronze Age dwelling platforms of north Humberside and those of Scandinavia⁵ suggesting a possible former cultural connection.

We would not like the foregoing arguments to be taken to imply that we necessarily prefer the hypothesis of human activity to that of climatic change in explaining the former occurrence of *Trapa* in north Humberside; we are simply suggesting that minds should be kept open. Still less would we like it to be thought that we are necessarily against the idea of a climatic optimum; we simply suggest that the new British *Trapa* records may not demand this as an explanation.

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R factor transfer *in vivo* in sheep with *E. coli* K12

It has been inferred that the bacterium *Escherichia coli* K12 is a naturally "feeble" strain which can be used in some genetic engineering experiments with little concern that it might "escape" containing a reconstituted plasmid. Also, there seems some doubt as to whether *in vivo* plasmid transfer, especially from the commonly used *E. coli* K12 F⁻, can occur to a significant extent within the gastrointestinal tracts of normal, adult humans or other animals^{1,2}. This implies that even if a few organisms should be ingested by laboratory personnel, transfer of a plasmid to other bacteria would be negligible and the "feeble" *E. coli* K12 should be quickly eliminated from the human gastrointestinal tract.

In an attempt to ascertain how antibiotic-resistant organisms have become so widespread in farm livestock, I have studied the conditions under which R factors could be transferred from donor to recipient bacteria *in vivo* in sheep³. These experiments were repeated using donor and recipient strains of *E. coli* K12 with similar results. By imposing a short period without food (24–48 h duration) on these animals, the environment within the gastrointestinal tract was altered and the administered *E. coli* K12 strains were able to multiply *in vivo*, reaching a population density sufficient to donate or receive an R factor. An experiment was also conducted in which an R factor was transferred *in vivo* from a small number of administered *E. coli* K12 donor organisms (4.0×10^3 cells) to the natural coliform flora of an adult sheep where it survived for 7 d (in up to 10^5 wild-type cells per g faeces). In similar experiments transferring the same R factor from 'animal' donor strains to the natural microflora of sheep, coliform organisms containing the R factor were sometimes excreted for only a few days. At other times, however, these organisms could be excreted for weeks or even months afterwards. It is not known whether the particular donor organism has an effect on the duration of carriage in the microflora of an animal, but this seems unlikely.

These experiments were carried out in the absence of any form of antibiotic treatment, and possession of the R factor should have conferred no special survival value on the host bacterial cells. Using similar nutritional conditions, therefore, it may be possible to transfer other plasmids, perhaps even reconstituted ones, *in vivo* in sheep.

If *in vivo* transfer can be accomplished in one animal species using

E. coli K12 organisms, it is likely to take place in other animal species, including humans. The difficulty will be to define the conditions in which it occurs.

Short starvation periods or other changes causing temporary alteration of the environment and microflora of the gastrointestinal tract may well potentiate plasmid transfer in monogastric as well as ruminant animals. It is in the interests of all concerned in genetic engineering that this possibility is recognised.

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Human ζ haemoglobin chain

THE embryonic ζ chain occurs in combination with γ chains in haemoglobin (Hb) Portland¹. Lehmann² and Huehns³ have suggested that the ζ chain is an α -type chain and attempted to confirm the idea by comparison of the amino acid compositions of the tryptic peptides from the ζ chain with those of the human α chain^{4,5} and known mammalian α chains⁶. From similar data other workers have proposed the same α chain relationship for the corresponding χ -chain found in other mammals⁷. The presence of a Tyr–Arg dipeptide^{4,5}, the absence of a D helix^{4,5}, the identification of residues which could participate in α – β contacts⁵ and cooperative oxygen binding by Hb Portland^{4–6} have been used to support the contention that the ζ chain is a primitive α chain.

I submit that the relationship between the ζ and α chains has not been clearly established by the published evidence. Tyr–His, present in non- α chains, could become Tyr–Arg as a result of a single base change. The missing D helix, like the detection of appropriate α – β con-

tacts, could easily result from the deliberate attempt to align the ζ -tryptic peptide compositions with the α -chain sequence. Certainly the β_4 tetramer does not show cooperative oxygen binding but this does not preclude the possibility that some asymmetric tetramers with no α -chain content could have cooperative function.

Examination of the amino acid compositions of the various human Hb chains¹ by any of the proposed methods^{8–10} suggests that the ζ chain is most closely related to the γ chain. The available tryptic peptide compositions of the ζ chain can be aligned with the γ -chain sequence to give 46 differences in 131 positions of comparison (the alignment includes a D helix). In the most recent comparison of ζ -chain peptide compositions and the α -chain sequence there were 47 differences in 135 positions.

I do not claim that the ζ chain is closely related to the γ chain. I do suggest that the lack of restraints in aligning tryptic peptide compositions within a family of related sequences can lead to erroneous and misleading conclusions, and urge that at least the tryptic peptide sequences be obtained before the principle of least dissimilarity is invoked and evolutionary or functional relationships proposed.

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¹⁰ Harris, C. E., and Teller, D. C., *J. theor. Biol.*, 38, 347–362 (1973).

LEHMANN REPLIES—The α and γ chains of man have more than 60% of their sequences in common. It is therefore not surprising that it is possible to align parts of the ζ chain with either. The fact remains that haemoglobin (Hb) Bart's (γ_4) shows no cooperativity, whereas Hbs F ($\alpha_2\gamma_2$) and Portland ($\zeta_2\gamma_2$) do. The cooperativity of Hb Portland is such¹ that it is difficult to visualise it without the conventional $\alpha_1\beta_2$ contacts typical of cooperative haemoglobins. The fact that it is possible to align by homology residues of the tryptic peptides of the ζ chain in a manner which places $\alpha_1\beta_2$ contacts in the expected positions fully justifies that exercise.

- ¹ Tuchinda, S., Nagai, K., and Lehmann, H., *FEBS Lett.*, 49, 390–391 (1975).

Matters arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 300 words and the briefest of replies, to the effect that a point is taken, should be considered.

reviews

Diseconomics of deforestation

N. W. Pirie

Losing Ground: Environmental Stress and World Food Prospects. By Erik P. Eckholm. Pp. 223. (Norton: New York, April 1976.) Cloth \$7.95; paper \$3.95.

DESERTS are expanding, hillsides washing away, rivers and reservoirs are silting up and the oceans are becoming polluted. Eckholm argues that the destruction of forests is the main cause of all but the last of these misfortunes. He states a convincing case and points out that it is not new. Hadrian tried to protect the cedars of Lebanon, and, at the end of the eighteenth century, deforestation was blamed for flooding around Grenoble and in Alpine valleys. As a result, reforestation programs were started in the Alps and Appenines.

Iron smelting was responsible for the later stage of deforestation in Britain: agriculture was responsible for the early stages. Eckholm says that more forest is now being destroyed to make farm land than to supply industrial timber, that the swidden (slash and burn) technique destroys more trees than settled agriculture, and that half the wood cut throughout the world is needed for fuel. Destruction for domestic fuel is not new. In four years after the French Revolution, 3.5 million hectares of forest that had been protected by aristocrats was destroyed. Now, 20–30% of the family income in some countries is spent on fuel, and wood costs as much as the equivalent amount of kerosine.

Oil prices are not likely to diminish; the demand for wood fuel will therefore increase in the less developed parts of the world and make planned forestry even more important, both to maintain supplies of wood and to prevent erosion. A survey is quoted showing an annual loss of a few kilograms of soil from forested hillsides, a few tons from pasture, but 8–100 tons from arable land. There is, however, no hope of improvement in methods of land use until the informed consent of the inhabitants has been won. A similar point arises when overgrazing creates deserts. If, as is stated, the Sahel would produce twice as much meat and milk if it carried half the present number of cattle, it should be possible to convince people of this. Before the commons were enclosed,

our forefathers had controlled the number of animals each person had the right to graze. If we could do it, others can.

Eckholm does not want to preserve tropical rain forest for sentimental reasons, nor does he support the mistaken idea that forests are a better source of atmospheric oxygen than other forms of plant cover. But he quotes examples of the failure of attempts to replace it by 'western'-type farming, and argues that that approach is never likely to succeed. He is no more hopeful about the more sophisticated forms of swidden that are grouped under the term 'corridor' systems.

Clearly, the ground should seldom, if ever, be left bare. This means that perennial crops will be the mainstay of satisfactory farming in rain forests. Rubber and oil palms are traditional. The products from these crops, as of many other tropical crops, move into world trade; a variable, but usually small, fraction of the money earned moves back to the primary producers.

They would fare better if they could grow crops that yield a balanced diet for local use. There will be a revolution in agriculture in the wet tropics when perennial plants are developed from which food with a reasonable protein content can be prepared by simple techniques.

This is a popular account of our mismanagement of Earth's biological resources. British readers should remember that Americans think a 'watershed' is a valley; scientific readers may be irritated by the repetitions, the 'purple passages', and the author's ignorance of the meanings of such words as decimate, hopefully, ineluctable and sedimentation. Scientists will, however, welcome the impressive bibliography—30 close-packed pages of references and suggestions for further reading. □

Norman Pirie has recently retired as head of the department of biochemistry at Rothamsted Experimental Station, Harpenden, UK.

Forest energy for half the world

Forest Energy and Economic Development. By D. E. Earl. Pp. vii+128. (Clarendon: Oxford; Oxford University: London, October 1975.) £5.

WHEN we run out of oil and coal can we use wood? This small book, part of an Oxford series on Science Policy, will be a good start for debates on long-term substitutes for the alarmingly expensive and diminishing fossil fuels.

Dr Derek Earl's answer to the energy crisis is that replacement of fossil fuels by wood is feasible now in countries which have a low per capita demand for energy, a small population and plenty of forest land. Good planning and management are essential, for without control, wood is as temporary a resource as oil or coal. Many developing countries are heading the same way as Europe, which has long since destroyed much of its forests. In most of the richer, highly developed countries forest energy seems to offer little relief from the impending shortage of fuel unless the population is reduced and people can be persuaded

to want less energy, a task more appropriate to poets and philosophers than to forest economists.

The statement in the preface that "the world's forests incorporate solar energy into organic material . . . at an annual rate far in excess of the world's present economic needs" is shown later to be almost irrelevant compared with the practical tasks of growing, harvesting, processing, transporting and marketing forest fuels cheaply.

After preliminary chapters putting economic growth and energy resources into perspective, most of the book is devoted to ways of supplying firewood and charcoal for particular situations in developing countries, including several case studies from the author's own wide experience. He has a particular interest in the technology of charcoal production which is well described.

Half the wood harvested in the world is used for fuel and about half the people of the world depend more on wood and charcoal for their cooking and heating than on other sources of energy. Of the world's total recorded

annual consumption of energy from all sources, 7% is from wood. In such countries as the US and the UK, less than 1% comes from wood, but in Tanzania, Nigeria, Nepal and several other countries the proportion is over 90%.

The book is not difficult reading for the non-specialist as the author explains many of the basic concepts of both forestry and economics. His efforts to present ideas and data in a simple direct style, however, have resulted in a somewhat uncritical treatment.

As a source of data for discussion or decisions the book is only as good as its sources which the author acknowledges to include unreliable esti-

mates. Who can record accurately the 'theft' of firewood gathered by people who regard forests as God-given and bountiful? A determined survey of consumers in one country showed that only 1.5% of fuelwood used had been recorded previously in government trade statistics.

The renewability of forests is a key point in the book. At the common yield of about 3-6 tonnes per hectare per year fuel wood production has not often been a profitable use for land. The occasional yields of eucalypt plantations of more than 40 tonnes per hectare per year on the best sites, however, are highly profitable. Can the yield be increased economically on other sites?

The current low prices of fuel wood are partly based on the wrong assumption that a forest costs nothing to grow or replace. With this assumption wood is mined, dissipating the energy capital of forests. Growing fuel wood as a crop will become a legitimate use for valuable land as the price and undesirable social costs of oil and its substitutes rise with scarcity. This is Earl's economic theme, the effects of depletion and scarcity on the price of fuel.

Earl's book may be as he says the first to assess the potential of the world's forests "as major stores and suppliers of energy". It is unlikely to be the last on this increasingly urgent subject.

Ken Eldridge

Soviet geological cruises

Rift Zones of the World Ocean. Edited by A. P. Vinogradov and G. B. Udintsev. Pp. viii+503. (Halsted Wiley: New York and Chichester; Israel Program for Scientific Translations: Jerusalem, December 1975.) \$49.50; £24.75.

DON'T rush straight off to the bookshop, all of you eager to join in the IPOD research program. Although its title suggests a quick way of catching up on what went on in the ocean crust while you were doing your lunar rocks, this book is not that at all. First published in Moscow in 1972, with Soviet references up to 1970, and foreign references to about 1968, this is a compilation of Soviet articles about two geological research cruises in the Indian Ocean in 1964 and 1967.

I stress the dates because it is most striking as a historical document. At that time, there was an equivocal relationship between Soviet marine geology and plate tectonics. A year or so before, diverging arrows had begun to appear on Soviet diagrams of mid-ocean ridges, although the text was usually reticent about their precise significance. Here, the silence is profound in most of the book, most notably in the chapter on magnetism, but in the last chapter a clear acknowledgement of oceanfloor spreading as a possible process occurs both in a diagram and in the text.

Since most of the new ideas from this research have already found their way into the scientific consciousness, becoming transformed in the process, why should one buy the book? Not for its diagrams or the precision of its writing, both about par for Soviet texts. But the chapter on the 1967 seismology still looks modern, and there is one too on mineralisation in

which two small sulphide-bearing rocks are described. The large collection of ultramafic rocks is well described, and there are a fair number of major element analyses, but the usefulness of the book as a quarry for data is severely reduced by the lack of any detailed positions within each polygon for the dredge stations.

I'm sure that if Gleb Udintsev and his colleagues were writing it again now, they would write it very differently, so that it is hard to recommend it enthusiastically now as a newly published book.

J. R. Cann

Bio-inorganic chemistry

An Introduction to Bio-Inorganic Chemistry. Edited by David R. Williams. Pp. 402. (Thomas: Springfield, Illinois, January 1976.) \$24.50.

THE preface states that this book was "conceived in a bar on Amsterdam airport by an *ad hoc* committee of inorganic researchers delayed by a pilots' strike". Conception in such circumstances might well be expected to produce a somewhat unselected set of characteristics in the offspring. Now that the child has been delivered we can view the baby and, mercifully, although it has the predictable blemishes, it has many endearing features. The worst blemish it shares with its parents is the wish to be too many different things, clearly shown by the section headings: bio-inorganic chemistry; instrumental approaches to bio-inorganic chemistry; biomedical applications. Taking these in turn, we might have expected first, an orderly introduction to the subject but in its place we have a jumbled set of chapters, each good in its own right, on metal complex formation, oxygen and nitrogen, iron, calcium and magnesium, and on metal-

loenzymes but nothing on nucleotides sugars and membranes.

Section II is similarly disjointed. It is good on thermodynamic studies but there is no coverage of spectroscopic, magnetic, electron microscope probe methods, and so on. Bio-medical applications is a different brew but it has very little information on, for example, the effect on disease of element concentrations, although it does introduce many risky topics—for example, cancer, death, and so on (everlasting life escapes). This is a very complicated area of biological science and I am not sure that a superficial taste of some of it is not harmful or even addictive for readers never mind some of the authors.

In previous reviews of books on bio-inorganic chemistry, I have pointed to the absence of material on Na/K/Mg/Ca metabolism. This fault is remedied in this book. I now point to another defect of conventional bio-inorganic chemistry which is strikingly apparent here—the absence of reference to the role of non-metals, B, Si, P, S, Cl, Se, Br, I, and so on. This could easily be put right in a future edition.

The overall impression given by this book is of a subject, a very large subject, in the act of growing up. Bio-inorganic chemistry as seen here is beginning to receive an education, although it is still necessary for parts of it to learn their proper functions. Surely a major purpose of the book is achieved in directing attention to what inorganic chemistry is. Inorganic chemists must cease to occupy themselves with toy-rattles making noises about molecular spectroscopy and magnetism. They must again turn to the study of chemical properties. As this book shows, with its accent on enthusiasm, one big exciting area of inorganic chemistry is to be found within biology and much of importance remains to be discovered.

R. J. P. Williams

Carbonate petrology

Carbonate Facies in Geologic History. By James L. Wilson. Pp. xiv+471 (30 plates). Springer: Berlin and New York, 1975). DM 90; \$36.90.

Carbonate Facies in Geologic History is not written with the precision of Henry James, but neither is it as hard to read as *Finnegan's Wake*. Although several readings are required for understanding of many parts, this book is the kind of helpful work that would require close study even if the prose were pure.

Wilson's approach is inductive. He cites with approval Mark Twain's conviction, after viewing thousands of European cathedrals, that he should study another 97,000 or so before making up his mind on their aesthetic value. Geology, however, has always been the art of drawing sufficient conclusions from insufficient premises—the Samuel Butler view of life.

One of the biggest surprises is Wilson's decision (p10) to use Dunham's rather than Folk's classification "... to avoid the necessary rigidity involved in applying Folk's particle-type prefixes and because the packing concept of Dunham is important and should be designated by a primary name." I'm uncertain of Wilson's meaning, but for most carbonate petrologists the descriptive nature of Folk's classification and its applicability to environmental interpretation have made its use appropriate for many diverse problems.

The first chapter is a short outline of the principles of carbonate deposition. Chapter two reviews stratigraphic and facies patterns and paleotectonic settings. An outline of carbonate petrography is given in chapter three where useful descriptive techniques for environmental interpretations are stressed. The remaining chapters (4–11), except for a concluding summary (chapter 12), contain descriptions of many examples of marine carbonate facies.

In the descriptive chapters Wilson briefly outlines the tectonic setting, the geologic history, and the stratigraphy. Depositional and diagenetic factors that have led to the development of good porosity and permeability are stressed. Many characteristics of carbonate facies that are helpful in recognising favourable areas for petroleum exploration are included.

This book is of greatest value to carbonate petrologists engaged in petroleum exploration. The treatment is too detailed for undergraduate students, for whom it was not intended, and will even present difficulties for many graduate students and petroleum geologists for whom the book was

written. A considerable amount of experience in diverse marine carbonate sequences is required for comprehension, especially a good background in paleontology and carbonate petrography.

At \$36.90 no student will rise early, eat a roll, brush his teeth, and stroll to the bookstore for a copy. Not willingly, at any rate. Few teachers not independently wealthy will wend their way to the cashier with a copy. If the book sells well enough for the publisher to issue a paperback edition at half this price, many individuals will buy it because this is a useful volume. Wilson has had an outstanding career in carbonate petrology; much of his knowledge is presented here.

M. Dane Picard

Fluorescence spectroscopy

Biochemical Fluorescence: Concepts. Vol. 1. Edited by Raymond F. Chen and Harold Edelhoch. Pp. xii+408. (Dekker: New York, August 1975.) \$29.50.

FLUORESCENCE spectroscopy is to many biochemists a Jekyll and Hyde affair. The classical uses of the technique—for example, histological staining, estimation of metabolite concentrations and of enzymic activities—are simple in application, and the results obtained easy to interpret. This situation began to change in the 1950s with the increasing sophistication of solvent shift and polarisation studies, although most biochemists could still appreciate the conclusions of such studies and their relevance to biochemistry even if they did not understand the physical basis of the techniques. Over the past decade, however, the subject has undergone a further transformation such that totally new aspects—for example, circular fluorescence polarisation—are, at least within the confines of a scientific paper, beyond the grasp of most biochemists not working in the field.

This book will do a great deal to alleviate the problem. It consists of seven chapters, each devoted to a basic concept or application of fluorescence spectroscopy and written by one or more leading authorities in that area. The general topics covered are fluorescence decay, fluorescence polarisation, energy transfer and kinetic techniques utilising a change in the fluorimetric properties of the reactants. This volume is devoted solely to theory and instrumentation. The applications to biological systems will be covered in volume 2. Nonetheless, a biochemist interested in understanding the basis of fluorescence techniques could read

this book with advantage. Each topic is developed from a simple description of the physical processes involved to the level needed to understand the latest publications. The extent of the mathematical development included is just about right to maintain continuity without overburdening the reader who does not want a rigorous proof of each step. Such a treatment, necessarily, leaves many logical gaps which, if the book is well referenced, the interested reader can fill in himself. In places, the references of this otherwise useful book falls short of that which will enable him to do this easily. The extensive treatment of several areas beyond that found in the literature will make this an attractive book for workers in the fluorescence spectroscopy field. This is especially true of the Dale and Eisenger article on energy transfer which takes up two-fifths of the book and half of which is devoted to a graphical treatment of the vexed orientation factor.

The contributors—especially Steinberg—should be commended for their honesty in clearly stating the problems of applying the techniques to biological systems and in outlining the areas in which present theory does not adequately explain the experimental results.

M. T. Flanagan

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London's Zoo

London's Zoo. (An Anthology to Celebrate 150 Years of the Zoological Society of London, with its Zoos at Regent's Park in London and Whipsnade in Bedfordshire.) Compiled by Gwynne Vevers. Pp. 159. (Bodley Head: London, Sydney and Toronto, April 1976.) £4.95.

WHAT a great deal of fun Dr Gwynne Vevers must have experienced sifting through the enormous amount of material available to him and what frustrations he must have experienced when it came to editing, perhaps one should say culling, the plethora of quotable quotes that he must have gathered together. While reading the book one soon becomes aware of the careful thought which has gone into the form of presentation. Inevitably he has concentrated on the early days, days when the foundations of the Zoological Society of London were being laid with a clear emphasis on the advancement of zoological science and the development of a living collection of animals. In those days creatures arriving at the Society's premises might be found in a crate, a jar of spirit, or just a package of skin and skeleton for examination by the early zoological researchers. The Society's museum was consolidated in 1849 with the



Bear pit (1835) by George Scharf

Zoological Society of London (Michael Lyster)

Zoology Department of the British Museum, and since that date material available as a result of deaths in the animal collection has gone to this museum.

It was this insistence by the founding fathers on a scientific approach to the development of its collection that has resulted in the Society being held in the highest esteem in the zoological world. But to the millions of visitors who have visited the animal collection over the years it has been the "Zoo", a place to wonder at

strange creatures, to be amused, to recreate, to escape for a moment from the sometimes harsh realities of everyday existence.

The anthology is the Zoo itself, it involves animals and people, ideas and ideals, the serious, the flippant, the scientific and the anthropomorphic. It should make good and enjoyable reading for all who have a specific or passing interest in the growth and development of this great Zoological Society and its Zoo.

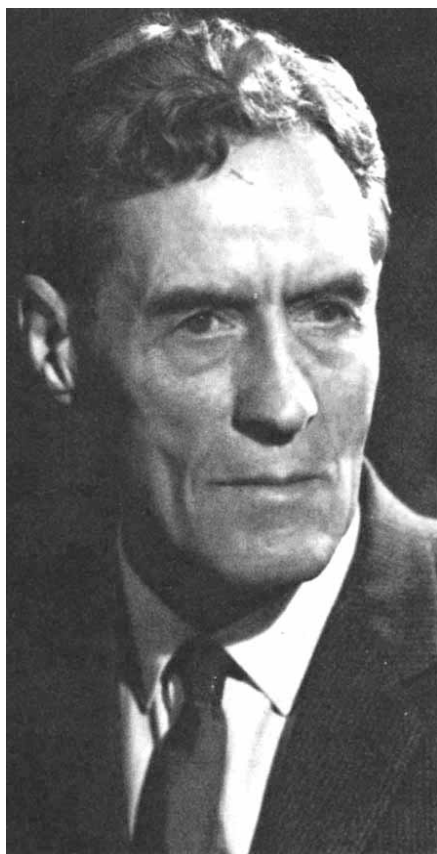
R. J. Wheeler

Lovell on Blackett

P. M. S. Blackett: A Biographical Memoir. By Sir Bernard Lovell. Pp. 115. (Royal Society: London, 1976.) UK £4; Overseas £4.10.

THIS book is a reprint of Bernard Lovell's account of Lord Blackett published recently in the *Biographical Notices of the Royal Society*. These *Notices* are accounts of recently deceased scientists written by their friends. They are of very great interest now and will be invaluable to historians in the future. Regrettably few libraries, especially in the US, subscribe to the series. The Royal Society wisely insists that the *Notices* be written within a few months of the subject's death and usually gets an account that bears the impress of friendship and of recent memories of personality and oddities of character.

It is not easy to write a biographical notice: on the one side there is the pressure of friends, relations, and often of the widow, to omit anything at all colourful (a widow once insisted that I omit the fact that a man had died of a stomach haemorrhage after a lunch



Lord Blackett

to celebrate his 90th birthday). On the other side there is the difficulty of giving a realistic account of scientific innovation and its attendant struggles, a thing done with such *éclat* by James Watson, and above all of letting the character emerge from action and anecdote rather than from explicit description.

In writing of Patrick Blackett all these difficulties are magnified by the immense achievement. The writer could easily be overwhelmed by the ramifications of the three careers, in science, in warfare and in politics and by the major achievements in three fields of science—nuclear physics, cosmic rays and geomagnetism. In fact Lovell has done it superbly well. The account is beautifully clear and readable, and Blackett emerges from it as the uniquely intelligent and humane figure that he was. It is a book that every young physicist should own and that no-one who knew Patrick could possibly be without. The immense care that has gone into getting it all right has been enormously worthwhile. This is how Blackett appeared to his contemporaries and anyone who writes about him in the future must rely largely on this.

Edward Bullard

obituary

Gordon Miller Bourne Dobson, CBE, ScD, FRS, former reader in meteorology in the University of Oxford, died at the age of 87 on March 10. After school at Sedbergh he went up to Gonville & Caius College, Cambridge. The post he held from 1911–1913, as student-assistant at Kew Observatory, followed by the Lectureship in Meteorology at the Central Flying School at Upavon, established the broad interest in the physics of the atmosphere which dominated his long and productive life. During his period at the Royal Aircraft Establishment, Farnborough, in the First World War, he was invited by Professor F. A. Lindemann to join him at the Clarendon Laboratory, Oxford, where he was appointed University Lecturer in 1920.

Their discovery, from the study of meteor trails, of a warm region in the atmosphere at heights of ~50km, and the inference that the source of heat must be the absorption of solar ultraviolet radiation by ozone, set Dobson on the researches which he carried out so energetically and fruitfully to the end of his life: the physics of the stratosphere in general and in particular the behaviour of ozone.

Fortunately Dobson has left us with a full and entertaining account of his own work up to 1966: Forty years' research on atmospheric ozone at Oxford: a history, *Applied Optics*, 7, 387–405 (1968). After that date he continued making substantial contributions to the study of atmospheric ozone, involving thousands of observations on 'No. 1', the prototype photoelectric photometer built with his own hands in 1927 and only slightly modified since (photomultiplier and electronics). His work was done almost

entirely at his home, and to a great extent financed out of his own pocket. There was a regular pattern of morning work, in the study if necessary but preferably in the workshop or observing hut, gardening in the afternoon (all weathers), further work after tea and reading (mainly scientific but in his last years general and voracious) after supper. If this regime sounds monastic, he was no hermit, and visitors to Watch Hill were always welcome; after tea or morning coffee Dobson was always eager to discuss some new observations, demonstrate some new apparatus, or discuss the recent literature.

His gift of going directly to the heart of a problem made him an outstanding speaker and his enthusiasm was infectious: his lectures on the atmosphere at the Clarendon Laboratory were always packed.

In the ozone community he seemed a permanent fixture and we are still staggered by his leaving us; for those who were privileged to work closely with him there are treasured memories and deep gratitude for his life and example.

C. D. Walshaw

Karl Weissenberg was born in Vienna, Austria on 11th June 1893 and attended many different schools in Austria, Germany and France before matriculating in Frankfurt a/Main at the age of sixteen and a half. He subsequently studied at the Universities of Vienna, Berlin and Jena, taking Mathematics as his main subject, with Physics and Chemistry as subsidiaries, touching also upon Law and Medicine. He became a Privat-Dozent and, later, Professor of Physics at the University of Berlin.

In 1922 he joined the research team of M. Polanyi at the Kaiser Wilhelm

Institute für Faserstoffchemie in Dahlen, Germany, and there, for a period of about six years, worked on X-ray crystallography. His fame in this field rests on his invention of a new X-ray goniometer, which allowed for the first time, a unique determination of 3-dimensional crystallographic structure. The idea was only slowly adopted, and it was not until after the war that the Weissenberg X-ray Goniometer, as it is known, came into general use. By 1929 his attention turned from crystallography to rheology and he presented a general analytical method for finding the shear rate at a capillary wall, now widely used.

Just before the war, Weissenberg took up residence in Britain, and his wartime work on flame thrower fuels stimulated his curiosity and interest in the so-called anomalous liquids, and hence in the field of liquid rheology itself. He also demonstrated, by means of rubber and fabric sheets, the importance of rotation of the embedded co-ordinate system in a flowing continuum. He had much earlier conjectured that certain fluids were elastic and now came the inference of the existence of a tension along the streamlines, since amply demonstrated. At this time, too, the essence of the Weissenberg Rheogoniometer was established. The principle behind this second major invention was that it allowed the stresses around the full solid angle in a flowing continuum to be experimentally determined.

In addition to a deep and sensitive appreciation of a range of natural phenomena, Karl Weissenberg was an entirely engaging and unselfish person. His accord with nature was faithfully mirrored in that with his fellow human beings. He was notable in his scientific achievements and noble in personal qualities.

John Harris

announcements

Awards

The American Society for Microbiology has awarded:

The **Wyeth Award** in Clinical Microbiology to **Edwin H. Lennette** of the California Department of Health.

The **Eli Lilly and Company Award** to **Ronald W. Davies** of Stanford University

School of Medicine.

The **Carski Foundation Distinguished Teaching Award** to **Elizabeth R. Hall** of Washington State University.

The Biochemical Society has given: The **BDH Award in Analytical Biochemistry** to Professor John Landon of St Bartholomew's Hospital.

The **Colworth Medal** to Dr W. J.

Brammar of Edinburgh University.

The **Keilin Medal** to Dr S. M. E. Magnusson of the University of Aarhus.

Meetings

July 7–9, **Annual Meeting of the Biochemical Society**, London (The Meetings Officer, The Biochemical Society,

7 Warwick Court, High Holborn, London WC1R 5DP).

June 23, **Nuclear Medicine Imaging Systems**, London (British Institute of Radiology, 32 Welbeck Street, London W1M 7PG).

October 12–15, **Evolution and Lateralisation of the Brain**, New York (Conference Department, the New York Academy of Sciences, 2 East 63rd Street, New York 10021).

August 30–September 3, **The Chemistry and Uses of Molybdenum**, Oxford (Molybdenum Conference, Climax Molybdenum Co. Ltd., Villiers House, 41/47 Strand, London WC2N 5JS, UK).

September 21–23, **Autumn Meeting**, Sheffield (Dr John F. Gibson, The Chemical Society, Burlington House, London W1V 0BN).

Reports and publications

Great Britain

Forestry Commission. Forest Mensuration Handbook. (Forestry Commission Booklet No. 39.) By G. J. Hamilton. Pp. 274. (London: Her Majesty's Stationery Office, 1975.) £4.00 net.

HMSO Subject Catalogue No. 1. Statistics. (Publications of UK Government Departments and International Organisations. Pp. iii + 55. (London: HMSO, 1975.)

Universities Research Reactor. Annual Report, 1974–75. Pp. iii + 36. (Warrington: Universities Research Reactor, Risley, Warrington, 1976.)

University of Glasgow. The Hannah Research Institute: for Studies Relating to the Production and Utilization of Milk. Report 1975. Pp. 71. (Ayr: The Hannah Research Institute, 1976.) £1.00.

The Year Book of the Royal Society of London 1976. Pp. 425. (No. 80). (London: The Royal Society, 1976.) £3.20.

Department of the Environment. Sixteenth Progress Report of the Standing Technical Committee on Synthetic Detergents. Pp. v + 20. (London: HMSO, 1976.) 65p net.

Royal Observatory, Edinburgh. Annual Report, 1974/1975. Pp. 29. (Edinburgh: Royal Observatory, 1976.)

Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 273, No. 926: The Postcranial Skeletons of the Triassic Mammals *Eozostrodon*, *Megazostrodon* and *Erythrotherium*. By F. A. K. Jenkins and F. R. Parrington. Pp. 387–431 + Plates 1–6. UK £3.45; Overseas £3.55. Vol. 273, No. 927: A Discussion on Water Relations of Plants. By J. E. Monteith and P. W. Weatherley. Pp. 433–618. UK £9.75; Overseas £10. (London: The Royal Society, 1976.)

Agricultural Development Advisory Service—Ministry of Agriculture, Fisheries and Food. Wildlife Conservation in Semi-Natural Habitats on Farms: A Survey of Farmer Attitudes and Intentions in England and Wales. Pp. viii + 69. (London: HMSO, 1976.) £1.25 net.

Scottish Development Department. Towards Cleaner Water 1975—Report of a Second Rivers Pollution Survey of Scotland. Pp. 72 + maps A–G. (Edinburgh and London: HMSO, 1976.) £5.90 net.

British Antarctic Survey. Scientific Reports. No. 54: The James Ross Island Volcanic Group of North-East Graham Land. By Dr P. H. H. Nelson. Pp. 62 + 11 plates. £6 net. No. 90: Measurements of Atmospheric Ozone at the Argentine Islands and Halley Bay, 1957–72. By J. C. Farman and R. A. Hamilton. Pp. 40. £2.90 net. (Cambridge: British Antarctic Survey, 1975.)

Other countries

Energy: The Case for Conservation. By Denis Hayes. (Worldwatch Paper No. 4.) Pp. 77. (Washington, DC: Worldwatch Institute, 1976 Massachusetts Avenue, N.W., 1976.) \$2.

Republic of the Sudan. Ministry of Agriculture—Agricultural Research Division. Annual Report of Yambio Research Station, 1963/1964. Pp. 19. (Wad Medani: Gezira Research Station, 1975.)

CERN—European Organization for Nuclear Research. CERN 75–20: The Design of the Control System for the SPS. By M. C. Crowley-Milling. Pp. 35. (Geneva: CERN, 1975.)

Royal College of Forestry, Stockholm. Studia Forestalia Suecica. No. 127: A Comparative Study of Chloroplast Morphogenesis in Seedlings of Some Conifers (*Parix decidua*, *Pinus sylvestris* and *Picea abies*). By Björn Walles and Jan Hudak. Pp. 21. No. 128: Influence of Photo- and Thermoperiod on the Initial Stages of Frost Hardening and Dehardening of Phytotron-Grown Seedlings of Scots Pine (*Pinus*

Person to Person

Wanted: A copy (preferably hard-back) of 'The Discovery of Time' by Toulmin and Goodfield (Hutchinson, 1965). Please contact Dr Peter J. Smith, Department of Earth Sciences, The Open University, Walton, Milton Keynes MK7 6AA, England, giving price, condition, etc.

The New York Academy of Sciences seeks, by July 1, nominations for its annual awards from individuals or organisations. For further information write to: The Chairman of the awards committee, The New York Academy of Sciences, 2 East 63rd Street, New York, New York 10021.

A symposium entitled, 'Pesticide Induced Delayed Neurotoxicity', jointly sponsored by the US Environmental Protection Agency and the US National Institute of Environmental Health Sciences was held in Washington, DC, February 19–20, 1976. The full text of the papers and ensuing discussions of the basic scientific and regulatory aspects of organophosphate-induced delayed neurotoxicity are to be made available free to interested individuals and institutions. All inquiries should be addressed to Dr R. L. Baron, Health Effects Research Laboratory, Environmental Protection Agency, Research Triangle Park, North Carolina 27711.

The André Lightwiz prize (8,800 francs) is awarded to French or foreign scientists who have specially distinguished themselves in research on calcium and phosphorus metabolism, whether in the clinical or more fundamental fields. Conditions of entry for the 1976 prize can be obtained from INSERM. Applications must reach the Director of the INSERM (c/o Dr Lucienne Bonnot, 101 rue de Tolbiac, 75645 Paris cedex 13, France) by June 30, 1976.

There will be no charge for this service. Send items (not more than 60 words) to Martin Goldman at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

silvestris L.) and Norway Spruce (*Picea abies* (L.) Karst.) By Aron Aronsson. Pp. 20. No. 129: Estimating the Accuracy of Site Index Curves by Means of Simulation. By Björn Hagglund. Pp. 34. (Stockholm: Royal College of Forestry, 1975.)

Energy, Mines and Resources Canada. Geological Survey of Canada. Paper 76–1A: Report of Activities, Part A. Pp. ix + 522. (Ottawa: Information Canada, 1976.) \$6.

Energy from Thermonuclear Fusion. Pp. 48.

(Garching bei München: Max-Planck-Institut für Plasmaphysik, 1976.)

Some Aziridines, N-, S- & O-Mustards and Selenium. (IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man, Vol. 9.) Pp. 268. (Lyon: International Agency for Research on Cancer, 1975. Distributed by World Health Organization, Geneva and HMSO, London.) Sw.fr.27.

United States Department of the Interior: Geological Survey. Bulletin 1388: Influence of Rainfall and Ancient Landslide Deposits on Recent Landslides (1950–71) In Urban Areas of Contra Costa County, California. By T. H. Nilsen and B. Turner. Pp. iii + 18 + plates 1 and 2. Professional Paper 774–C: Mount Abbot Quadrangle, Central Sierra Nevada, California—Analytic Data. By John P. Lockwood. Pp. iii + 18. (Washington, DC: US Government Printing Office, 1975.)

Biological Peroxidation of Lipids and Membranes. By M. Wolman. (Supplement to Vol. 11, *Israel Journal of Medical Sciences*.) Pp. 248. (Jerusalem: Israel Journal of Medical Sciences, 1975.)

Geophysical Institute, University of Alaska. Annual Report 1974/1975. Pp. 183. (Fairbanks, Alaska: Geophysical Institute, The University, 1975.)

United States Department of the Interior: Geological Survey. Professional Paper 726–E: Gravity and Aeromagnetic Study of Part of the Yakima River Basin, Washington. By S. L. Robbins, R. J. Burt and D. O. Gregg. Pp. iii + 7 + plate 1. Professional Paper 743–D: Dimorphism in Two New Genera of Devonian Tabulate Corals. By William A. Oliver, Jr. Pp. iii + 11 + plates 1–7. (Washington, DC: US Government Printing Office, 1975.)

Canada: Department of Energy, Mines and Resources. Geological Survey of Canada. Bulletin 246: Sedimentology of the Archean Yellowknife Supergroup at Yellowknife, District of Mackenzie. By John B. Henderson. Pp. ix + 62 \$6. Bulletin 247: Triassic Rocks of the Rocky Mountain Foothills and Front Ranges of Northeastern British Columbia and West-Central Alberta. By D. W. Gibson. Pp. 61 (9 plates). \$5. Bulletin 251: Investigations of Lower Paleozoic Geology, Foxe Basin, Northeastern Melville Peninsula, and Parts of Northwestern and Central Baffin Island. By H. P. Trettin. Pp. 177. \$10. (Ottawa: Information Canada, 1975.)

United States Department of the Interior: Geological Survey. Professional Paper 729–D: Geology of Sedimentary Rocks in Southern Yellowstone National Park, Wyoming. (Geology of Yellowstone National Park.) By J. D. Love and W. R. Keifer. Pp. x + 60. (Washington, DC: US Government Printing Office, 1975.)

University of California. Lawrence Berkeley Laboratory. Research Highlights, Fiscal 75. Pp. 75. (Berkeley, California: Lawrence Berkeley Laboratory, 1975.)

US Department of Health, Education and Welfare. Health Manpower in the Changing Australian Health Services Scene. By Ruth Roemer and Milton J. Roemer. Pp. viii + 87. (Springfield, Virginia: National Technical Information Service.)

Environment Canada: Fisheries and Marine Service. Technical Report No. 584. Intestinal Trematode Parasites (Trematoda: Digenea) and Food of Yellowtail Flounder (*Limanda ferruginea* (Storer 1839)) from the Scotian Shelf and Gulf of St. Lawrence. By J. S. Scott. Pp. iii + 12. Technical Report No. 593. Digenean Trematode Parasites and Food of Witch Flounder (*Glyptocephalus cynoglossus* L.) from the Scotian Shelf and Gulf of St. Lawrence. By J. S. Scott. Pp. iv + 9. Technical Report No. 594. Catch Statistics for the Bay of Fundy Herring Fisheries 1963–1974. By D. S. Miller and T. D. Iles. Pp. iii + 75. (St. Andrews, N.B.: Department of the Environment Fisheries and Marine Service, 1975.)

Smithsonian Contributions to Zoology, No. 203. New Species of Marine Molluscs from Pitcairn Island and the Marquesas. By Harald A. Rehder and Barry R. Wilson. Pp. iv + 16. (Washington, D.C.: Smithsonian Institution Press, 1975. For Sale By US Government Printing Office.)

Department of Energy Mines and Resources: Geological Survey of Canada. Paper 74–47. Upper Paleozoic Rocks of the Atlin Terrane, Northwestern British Columbia and South Central Yukon. By J. W. H. Monger. Pp. vi + 63 + 5 maps. (Ottawa: Information Canada 1975.) \$3.50; other countries \$4.20.

United States Department of the Interior: Geological Survey. Bulletin 1383–B: Upper Pleistocene Pyroclastic-Flow Deposits and Lahars South of Mount St. Helens Volcano, Washington. (Geology of Mount St. Helens Volcano, Washington.) By Jack H. Hyde. Pp. iv + 20. (Washington, DC: US Government Printing Office, 1975.)

Anuario del Observatorio Astronómico de Madrid para 1976. Pp. 422. (Madrid: Observatorio Astronómico, 1975.)

Criterios Sobre Política Científica/Criterios on Science Policy. (International Meeting on the Organization of Science, Madrid, 11–14 de Abril 1973. Actas Completas.) Edición preparada por Roman de Vicente Jordana. Pp. 271. (Madrid: Consejo Superior de Investigaciones Científicas, 1975.)

National Research Council Canada. Associate Committee on Scientific Criteria for Environmental Quality—Status Report. Pp. 68. NRCC No. 14109: Waste Heat in the Aquatic Environment. By D. R. Dickson. Pp. 39. \$1. (Ottawa: Publications, National Research Council Canada, 1975.)

Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft, Berlin-Dahlem. Heft 166: Resistenzbildung gegen systemische Fungizide (Benzimidazol-derivate) bei Colletotrichum Lindemuthianum (Sacc. et Magn.) Bri. et Cav. Von Dr Ehler Meyer. Pp. 135. (Berlin-Dahlem: Biologisches Bundesanstalt für Land- und Forstwirtschaft, 1976.) DM 16.60.